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This document summarizes the physical/chemical properties, toxicological information and environmental fate and effects of PCBs, with emphasis on its aquatic behavior. From these data criteria are developed for the protection of aquatic life.

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CRITERIA DOCUMENT

PCBs

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APPENDIX B. The Toxicity of Polychlorinated Polycyclic Compounds and Related Chemicals (Kimbrough).

APPENBIX C. Review of PCB Levels in the Environment (U.S. EPA, Office of Toxic Substances, Draft report).

APPENDIX D. Review of Carcinogenic and Co-Carcinogenic Effects of PCBs.

APPENDIX E. Metabolism and Biodegradation.

Errata
PCB Criteria Document

Sec. III.6.4 Effects on reproduction of birds

Line 3 should read - ppm of PCBs, corresponding to residues of only 1-2 ppm in whole eggs (150).

Sec. V. Criteria Formulation

Paragraph 1, line 5 should read - affected fish thyroid (73,95). At the 0.5 ppm dietary level PCBs caused enzyme induction in rats (219).

p. R-8

Reference 69: Pages cited should be 43-49 not 57-64

p. R-18

Reference 197: Pages cited should be 347-350 not 332-333

Appendices A and B are copyrighted materials and therefore have been omitted.

I. INTRODUCTION

I.1 Principal sources of information

Comprehensive reviews of the occurrence of polychlorinated biphenyls (PCBs) in the environment and their potential hazards were published in 1972 by an Interdepartmental Task Force (ITF) (1) and by a Panel on Hazardous Trace Substances (PHTS) convened by the Office of Science and Technology (OST) and the National Institute for Environmental Health Sciences (NIEHS) (2). The latter report formed the basis for a draft Scientific and Technical Assessment Report (STAR) prepared by the Office of Research and Development (ORD) of the Environmental Protection Agency (EPA) in September 1974 (3). More specialised reviews of the chemistry and toxicology of PCBs were published in 1974 by Hutzinger, Safe and Zitko (4) and Kimbrough (5): these reviews are attached as Appendices A and B respectively to this document. A draft report (6) reviewing the occurrence and levels of PCBs in the environment has been prepared by EPA's Office of Toxic Substances (OTS) in January 1976 and is attached as Appendix C. A summary of industrial uses and environmental distribution of PCBs in the United States was prepared for OTS by Versar, Inc., in February 1976 (7). Important collections of scientific data are included in the proceedings of two conferences on PCBs, held in 1971 and 1975 (refs. 8 and 9).

I.2 Uses and releases of PCBs into the environment

Polychlorinated biphenyls (PCBs) are manufactured by the direct chlorination of biphenyl. Commercial products are complex mixtures of chlorobiphenyls and are marketed for various uses according to the percentage of chlorine in the mixture. The major producer of PCBs in the United States is the Monsanto Company, which now markets four mixtures (with 21%, 41%, 42% and 54% chlorine) for closed electrical system uses under the trademark 'Aroclor'. Prior to 1970 Monsanto marketed a wider range of mixtures (containing up to 68% chlorine) and these were used in a number of other applications, including uses as plasticizers, heat transfer fluids, hydraulic fluids, fluids in vaccuum pumps and compressors, lubricants, wax extenders, etc. Following the discovery in the late 1960's that PCBs had become widespread environmental contaminants, Monsanto voluntarily limited its range of products and restricted sales to those for closed electrical system uses. However, because of the long life of many products containing PCBs, it is believed that a substantial fraction of the PCBs manufactured before 1971 remains in service (7). There is thus a potential for their discharge into the environment when the components containing them are scrapped or leak. Moreover, although imports of PCBs into the United States are small, comprising only 1-2% of domestic sales (7), much of the imported material appears to be sold for non-closed system uses (7). Thus, in evaluating the potential impact of PCBs released into the environment, it is necessary to consider not only the mixtures currently manufactured and sold by Monsanto, but also the other mixtures manufactured by Monsanto prior to 1970, and products manufactured overseas. Recent surveys have identified a number of sources of PCBs into the aquatic environment, including not only effluents from the manufacturing plant and the electrical industry, but also sewage treatment plants, urban run-off, steel and aluminum foundries, pulp and paper mills, automotive and metal finishing plants, hydraulic and heat transfer systems, investment casting operations, and shipping (10-12).

I.3 PCB mixtures and contaminants: the problem of evaluation

Evaluation of the potential environmental impact of the various commercial mixtures is complicated by the fact that the components of the mixtures differ greatly in their physical, chemical and biological properties. Accordingly the constitution of the mixtures often changes as the materials are transported through the environment, so that plants, animals, and humans are exposed to mixtures whose constitution differs from those whose toxicity has been tested in laboratory experiments. Although the environmental behavior and biological activity of a number of individual chlorobiphenyl isomers have been studied in recent years, it is still difficult to evaluate the potential toxicity of the complex mixtures found in the environment. In making this evaluation it is necessary to weigh carefully the results of studies of individual compounds, and to compare critically the environmental and toxicological properties of the commercial mixtures.

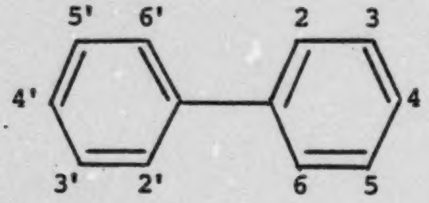
A further complication is that several commercial PCB mixtures have been reported to contain small quantities of highly toxic contaminants, polychlorinated dibenzofurans (PCDFs). Some of the toxic effects observed in animals and humans exposed to PCBs appear to be attributable to PCDFs, while others appear to be caused by PCBs themselves. There is also some evidence that small quantities of PCDFs may be formed from PCBs either in service or during metabolism by animals. Accordingly it is necessary to consider the potential effects of both classes of compounds when weighing the potential impact of PCBs released into the environment.

II. CHEMICAL AND PHYSICAL PROPERTIES

II.1 Nomenclature of chlorobiphenyls and isomers

Polychlorinated biphenyls consist of a mixture of chlorinated biphenyls with varying degrees of chlorination and isomeric constitution.

Some confusion has appeared in the literature because of the nomenclature that is used to describe the commercial preparations and the individual authenticated chlorobiphenyls. In order to clarify the nomenclature to be used herein, it is appropriate to digress briefly as follows. The biphenyl molecule has a total of ten (10) carbon-hydrogen bonds at which chlorine substitution can be accommodated. A schematic representation of the biphenyl molecule with the various positions at which substitution can be accomplished numbered in the American Chemical Society standard notation is presented below:



ACS Convention for Numerical Assignments
of Biphenyl Substitution

In the interest of a common usage, the following rules will be followed in this report:

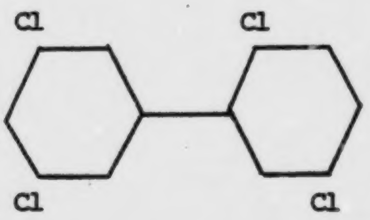
a. When referring to a mixture of different species, as occurs in the commercial products, the term polychlorinated biphenyls (abbreviation, PCBs) will be used.

b. Those species of chlorinated compounds that arise from a specified number of chlorine substituents on the biphenyl molecule will be referred to as chlorobiphenyls with a suitable numerical prefix to define the number of substituted chlorines; i.e., dichlorobiphenyl. Thus, there are a total of ten (10) chlorobiphenyls that might appear in the commercial mixtures.

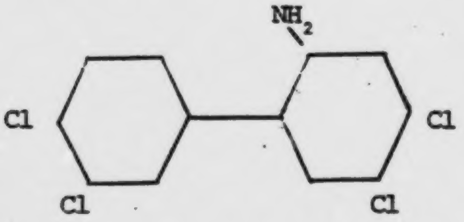
c. Those specific compounds that represent the class of compounds formed by a specific number of substituent chlorine atoms but differ in the locations at which substitution has taken place are referred to as isomers.

Thus, in terms of the above, the proper manner of referring to a commercial PCB mixture is as a "mixture of chlorobiphenyls containing various proportions of the isomers of each".

To illustrate the utility of the numbering system indicated previously, the correct names of the compounds shown below are:



2,3',5,5'-tetrachlorobiphenyl



3',4,4',5-tetrachloro-2-biphenylamine

In addition to biphenyl itself, which is found in some commercial mixtures, there are ten chlorobiphenyls comprising 209 possible isomers (Table II.1.1.)

In some sections of this report, chlorobiphenyls with 5 or more chlorine atoms will be referred to as "higher chlorobiphenyls" and those with 3 or fewer chlorine atoms will be referred to as "lower chlorobiphenyls". This distinction is made in recognition of the fact that the former group of compounds is much more persistent in the environment than the latter group, whereas tetrachlorobiphenyls are intermediate in persistence (Section IV.1 below).

II.2 Manufacture and nomenclature of commercial PCB mixtures

The world's principal manufacturers of PCBs are listed in Table II.2.1, with the trade names under which the products are sold. In the United States the sole manufacturer is the Monsanto Company which sells four mixtures under the tradename Aroclor$^{(R)}$. These four products, together with five mixtures whose manufacture has been discontinued since 1970, are listed in Table II.2.2 (from ref. 13). Except for Aroclor 1016, the last two digits in the Aroclor number specify the percentage of chlorine in the mixture. Aroclor 1016, introduced in 1971, is similar in constitution to Aroclor 1242 but is modified (by distillation) to include only about 1% of pentachlorobiphenyls (13).

II.3 Constitution of Aroclor mixtures

The commercial process by which the PCBs are made involved the chlorination of biphenyl with anhydrous chlorine in the presence of a catalyst which may be either iron filings or ferric chloride. The crude product is generally purified to remove color, traces of hydrogen chloride,

Table II.1.1

Empirical Formulation, Molecular Weights and Chlorine Percentage in PCBs

Empirical formula chlorobiphenyls	Molecular weight*	Percent chlorine*	No. of isomers
$C_{12}H_{10}$	154	0	1
$C_{12}H_9Cl$	188	18.6	3
$C_{12}H_8Cl_2$	222	31.5	12
$C_{12}H_7Cl_3$	256	41.0	24
$C_{12}H_6Cl_4$	290	48.3	42
$C_{12}H_5Cl_5$	324	54.0	46
$C_{12}H_4Cl_6$	358	58.7	42
$C_{12}H_3Cl_7$	392	62.5	24
$C_{12}H_2Cl_8$	426	65.7	12
$C_{12}HCl_9$	460	68.5	3
$C_{12}Cl_{10}$	494	79.9	1

*Based on Cl^{35}

Table II.2.1

The World's Major Producers of PCBs

Producer	Country	Tradename
Monsanto	U.S.A. and Great Britain	Aroclor(R)
Bayer	Germany	Clophen(R)
Prodelec	France	Phenoclor and Pyralene(R)
Caffaro	Italy	Fenclor
Kanegafuchi*	Japan	Kanechlor(R)
Mitsubishi-* Monsanto	Japan	Santotherm(R)
Sovel	U.S.S.R.	
Chemko	Czechoslovakia	

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* Production discontinued for domestic use.

Table II.2.2

AROCLOR PRODUCTS MANUFACTURED IN THE U.S.

Current	Discontinued	Percent Chlorine
1221		21
	1232	32
1016		41
1242		42
	1248	48
1254		54
	1260	60
	1262	62
	1268	68
	1270	71

and the catalyst by treatment with alkali and subsequent distillation. The resulting product is then a more or less complex mixture of the chlorobiphenyls, the precise composition depending on the conditions under which chlorination was carried out.

II.3.1 Molecular composition

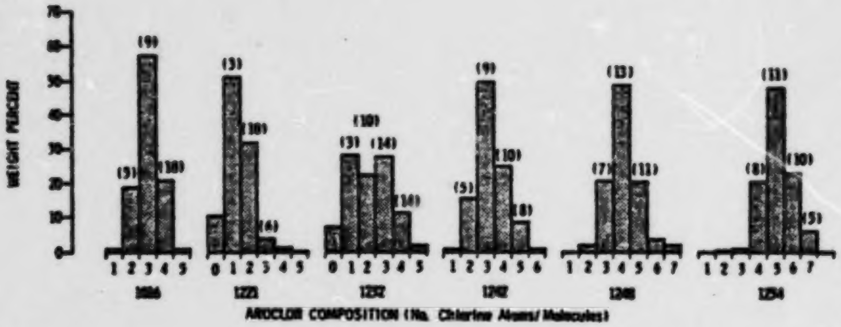
Table II.3.1 lists the distribution of the various chlorobiphenyls in six major Aroclor mixtures, as given by Monsanto (13), Webb and McCall (14), and Hirwe *et al*. (15). The differences between the results given for Aroclors 1242 and 1254 may reflect either differences in analytical methods or variations in constitution. These and other data are summarized in histogram form in Figure II.3.1 (16). No data appear to have been published on the molecular composition of the other Aroclor mixtures, but the data in Figure II.3.2 indicate that Aroclor 1262 consisted mostly of hexa- and heptachlorobiphenyls, and Aroclor 1268 mostly of octa- and nonachlorobiphenyls; Aroclor 1270 apparently consisted primarily of decachlorobiphenyl.

Figure II.3.2 presents high-resolution gas-liquid chromatograms of eight Aroclor mixtures (17). Each mixture contained a large number of components, including several isomers of each chlorobiphenyl; in most cases several major components are common to successive members of the Aroclor series.

Figure II.3.3 compares high-resolution gas-liquid chromatograms of Aroclors 1016 and 1242. The mixtures appear extremely similar except for the absence from Aroclor 1016 of a few minor constituents with relatively long retention times present in Aroclor 1242.

II.3.2 Isomeric constitution

A nearly complete identification of the chlorobiphenyl isomers present in Aroclors 1242, 1254, and 1260 was carried out by Sissons and



The congener composition of commercially available Aroclors based on the weight percent of biphenyls bearing different numbers of chlorine atoms/molecule. Data were obtained from reports by Webb and McCall (ref.20), Sissons and Welti (ref.18), and from information supplied by the Monsanto Industrial Chemicals Company.

Figure II.3.1 (from ref. 16)

Table II.3.1

Approximate molecular composition of Aroclors, from data in ref. 13 (M), ref. 14 (W), and ref. 15 (H).

Chlorobiphenyl	1221		1232	1016	1242			1248	1254			1260
	M	W	W	M	M	W	H	W	M	W	H	W
$C_{12}H_{10}$	11	7	6	Tr	Tr	-	-	-	Tr	-	-	-
$C_{12}H_9Cl_1$	51	51	26	1	1	1	Tr	-	Tr	-	-	-
$C_{12}H_8Cl_2$	32	38	29	20	16	17	4	1	0.5	-	-	-
$C_{12}H_7Cl_3$	4	3	24	57	49	40	39	23	1	-	0.5	-
$C_{12}H_6Cl_4$	2	-	15	21	25	32	42	50	21	16	36	-
$C_{12}H_5Cl_5$	0.5	-	0.5	1	8	10	14	20	48	60	45	12
$C_{12}H_4Cl_6$	-	-	-	Tr	1	0.5	-	1	23	23	18	46
$C_{12}H_3Cl_7$	-	-	-	-	Tr	-	-	-	6	1	1	35
$C_{12}H_2Cl_8$	-	-	-	-	-	-	-	-	-	-	-	6
$C_{12}H_1Cl_9$	-	-	-	-	-	-	-	-	-	-	-	-
$C_{12}Cl_{10}$	-	-	-	-	-	-	-	-	-	-	-	-

Tr - Trace (less than 0.1 percent)

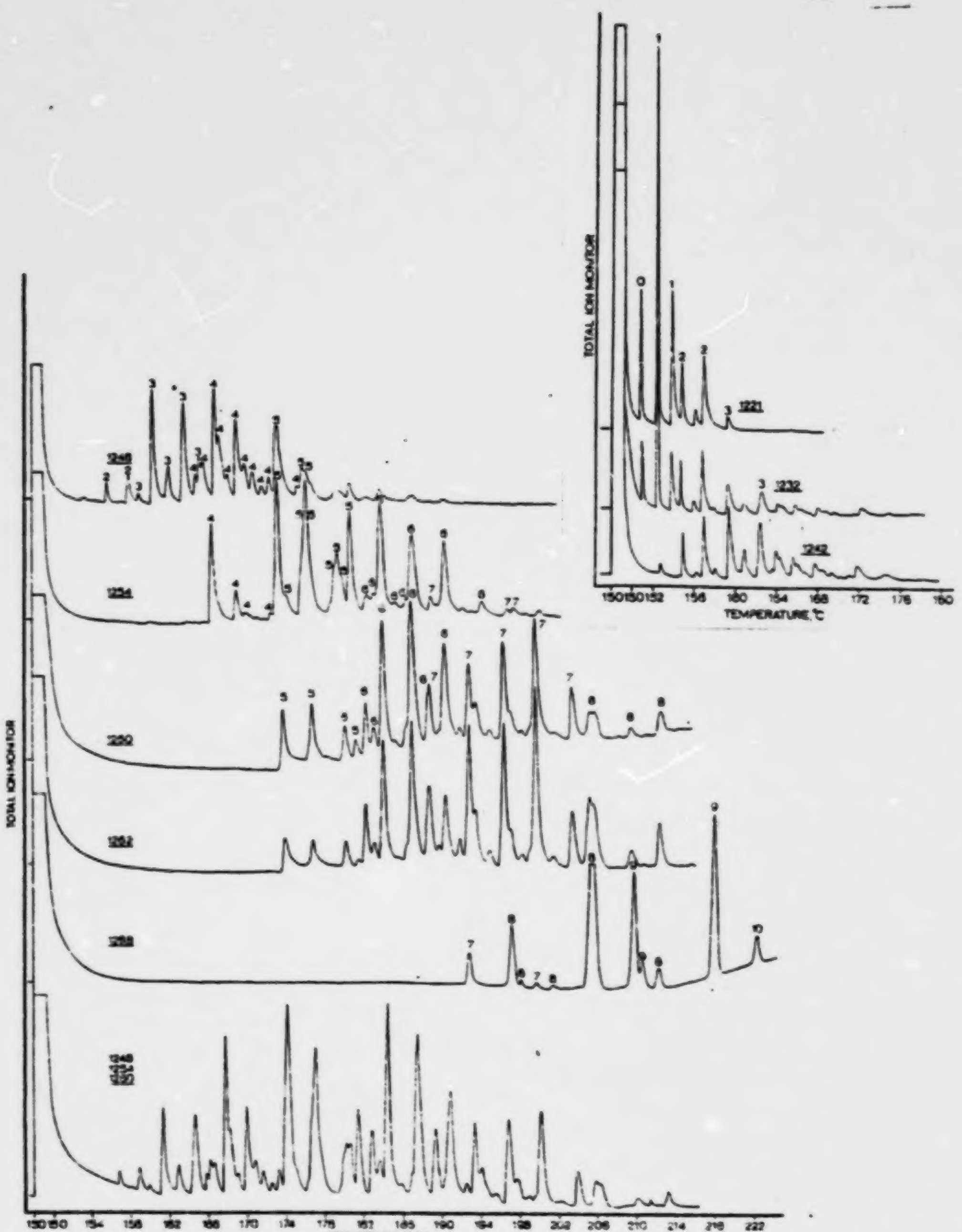


Figure II.3.2 Gas-liquid chromatograms of Aroclor mixtures (from ref. 17)

Underlined numbers designate the Aroclor formulation. Numbers above the peaks designate the number of chlorine atoms in the compound(s) determined from the mass spectrum; unnumbered peaks have the same number of chlorine atoms as marked peaks at the same temperature. Temperature program rate 2°C/minute, He 9 psig, 50 ft × 0.020 in. SE-30 SCOT column, PE-270 glc-MS.

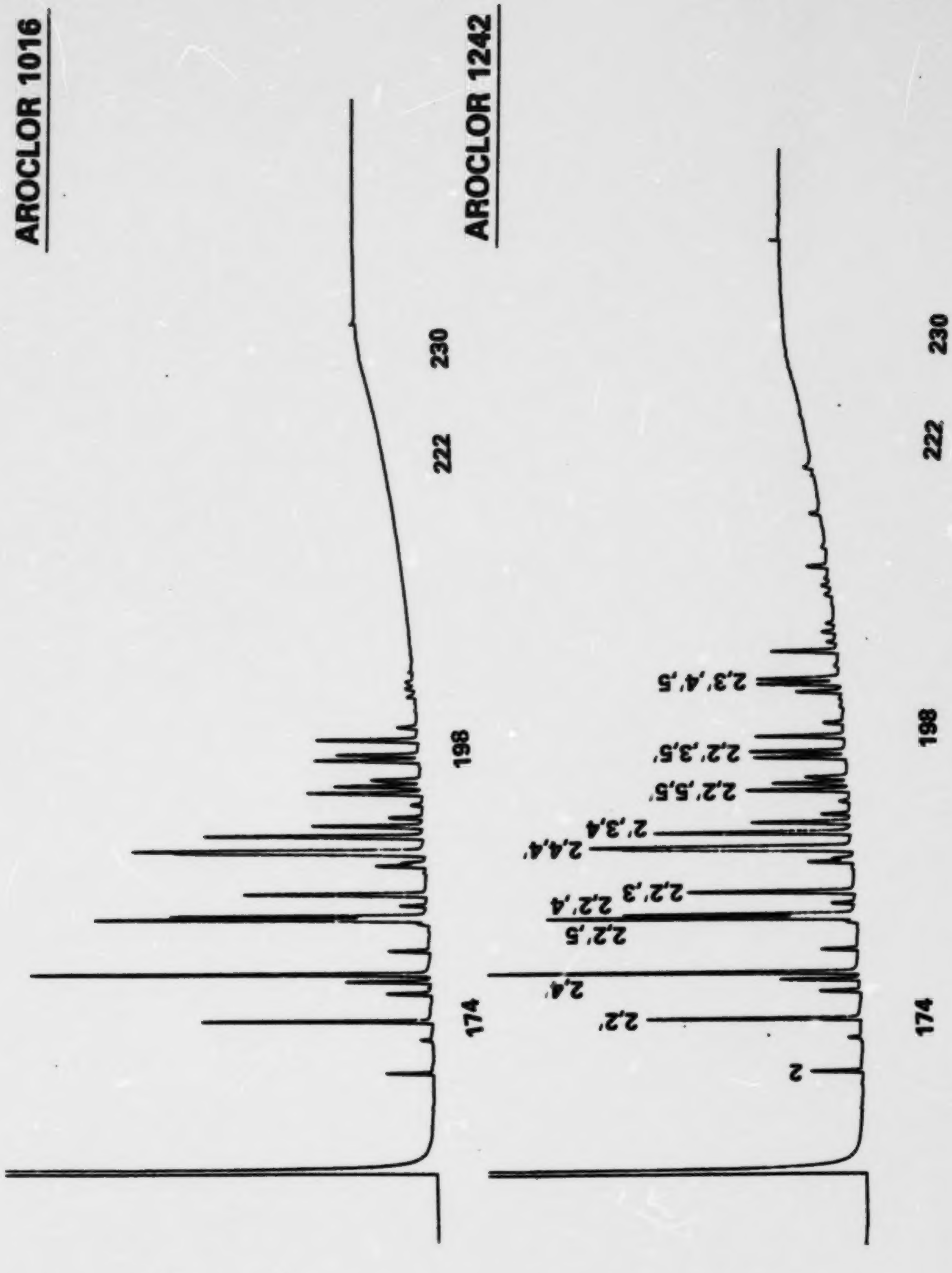


Figure II.3.3 High resolution capillary column chromatograms of Aroclors 1016 and 1242 (from ref. 13).

Welti (18) by use of a combination of gas-liquid chromatography, nuclear magnetic resonance (NMR) and mass spectroscopy (MS). The retention index (RI) system proposed by Kovats (19) was adopted in order to provide a characterization of the peak position in gas-liquid chromatography that is less sensitive to column conditions than retention time or relative retention time. Retention indices were used to identify minor constituents after the major constituents had been identified using NMR. The identifications of a number of the isomers have been confirmed independently (20).

Figure II.3.4 illustrates a gas-liquid chromatogram of Aroclor 1254 with the peaks numbered according to the scheme of Sissons and Welti (18). Table II.3.2 lists their identifications of the major constituents and Table II.3.3 lists the probable identities of the minor constituents. Figures II.3.5 and II.3.6 and Tables II.3.4 and II.3.5 give corresponding chromatograms and identification of the isomers present in Aroclors 1242 and 1260. Table II.3.6 gives the results of independent identification of the constituents of the major peaks in the chromatograms of Aroclors 1254 and 1260 (27): these agree closely with those in refs. 18 and 20.

II.4 Constitution of other commercial mixtures

Where data are available, the constitution of PCB mixtures manufactured in other countries appears to agree very closely with that of the corresponding Aroclor mixtures. Figure II.4.1, for example (from ref. 22) shows that gas-liquid chromatograms of Phenoclor DP6, Clophen A60, and Aroclor 1260 (all mixtures with 60% chlorine) are almost identical, except for a small difference in peak 3 in Clophen A60*. Jensen and Sundström (23) have identified and measured the relative quantities of most components of

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* This peak corresponds to components 11, 14, and 15 listed for Clophen A60 in ref. 23, and to components 40 and 41 listed for Aroclor 1260 in ref. 18. The difference appears to be the presence of 2,3,6,2',3',6'-hexachlorobiphenyl in Clophen A60, but not in Aroclor 1260 (18, 23).

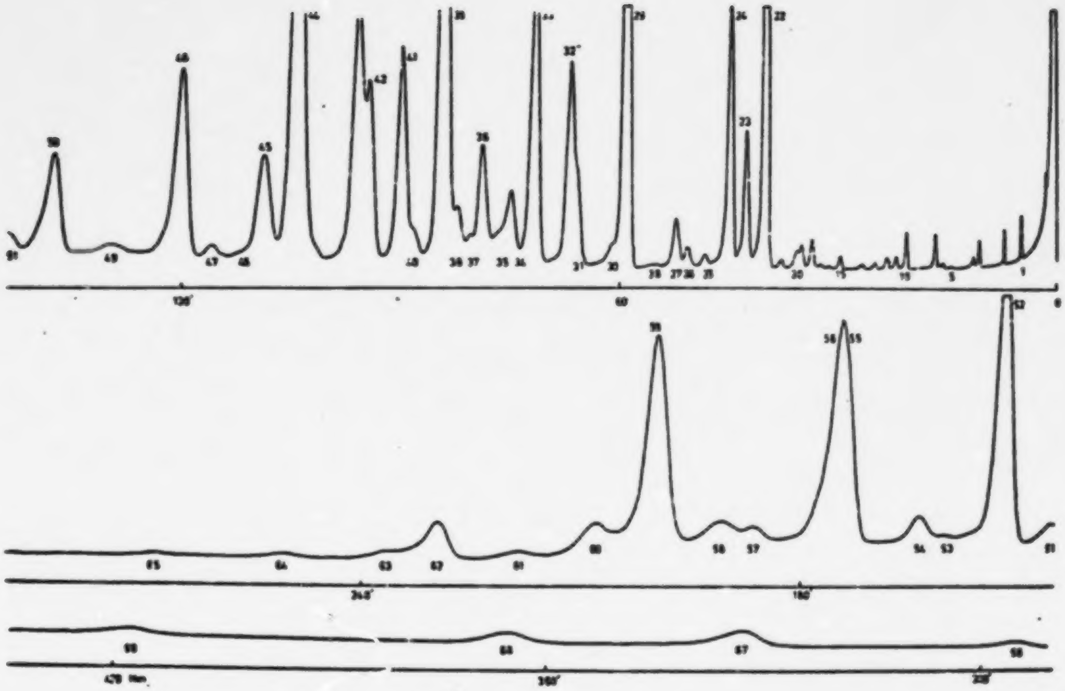

Figure II.3.4 Gas-liquid chromatogram of Aroclor 1254 (refs. 18, 21)

Table II.3.2 (from refs. 18, 21)

The retention indices, chlorine numbers and structures of the major PCB constituents in Aroclor 1254.

Peak No.	R.I.	Chlorine No.[a]	NMR determined structure	Alternative predictions	
				Structure	R.I.[b]
22	1994	4	2,5–2′,5′	2–2′,3′,5′	1906
23	2010	4		2–2′,4′,5′	2012
				2,4–2′,5′	2010
24	2022	4	2,3–2′,5′	3–2′,4′,6′	2021
				2,4–2′,4′	2027
		(5)		2,6–2′,3′,6′	2017
29	2089	5	2,5–2′,3′,6′	2,6–2′,3′,5′	2092
32	2119	5	2,3–2′,3′,6′		
33	2136	4	2,5–3′,4′		
36	2159	5		2,5–2′,3′,5′	2159
39	2175	5	2,5–2′,4′,5′	2,4–2′,3′,5′	2175
				3,5–2′,4′,6′	2175
		(6)		2,6–2′,3′,4′,6′	(2175)
				2,3,6–2′,3′,6′	2172
41	2191	5	2,4–2′,4′,5′	2,3–2′,3′,5′	2189
42	2203	5	2,3–2′,4′,5′		
43	2207	5	2,5–2′,3′,4′		
44	2228	5	3,4–2′,3′,6′	2,4–2′,3′,4′	2226
45	2238	—		2,3–2′,3′,4′	2240
				2,6–3′,4′,5′	2240
				3,4–3′,5′	2238
				2,3,5–2′,4′,6′	2240
48	2264	6	2,3,6–2′,4′,5′	2,4–2′,3′,4′,6′	(2263)
50	2299	6	2,3,4–2′,3′,6′		
52	2321	5	3,4–2′,4′,5′		
55	2356	5	3,4–2′,3′,4′		
56	2356	6	2,4,5–2′,4′,5′	2,5–2′,3′,4′,5′	(2360)
				3,5–2′,3′,4′,6′	(2357)
59	2390	6	2,3,4–2′,4′,5′	2,3–2′,3′,4′,5′	(3291)
				2,4,6–3′,4′,5′	2388
		(7)		2,3,5–2′,3′,5′,6′	(2390)

[a] Cl No. between brackets; Chlorine number of minor constituent not associated with accurate R.I.

[b] R.I. between brackets: Predicted R.I. using Table VIII of Ref18

Table II.3.3 (from ref. 18)

THE RETENTION INDICES, CHLORINE NUMBERS AND PREDICTED STRUCTURES OF THE MINOR PEAKS IN AROCLOR 1254

Peak No.	*R.I.*	*Chlorine No.*	*Structure*	*Predicted R.I.*^{a}
1	1490	0	Biphenyl	1491^b
2	1579	1	2-	1577^b
3	1672	2	2-2′	1669^b
4	1688	1	4-	1687^b
5	1750	2	2-5′	1754
6	1758	—	2,4-	1756^b
7	1763	2	2-3′	1770
8	1774	3	2-2′,6′	1765
9	1782	2	2-4′	1782^b
10	1833	3	2-2′,5′	1831
11	1849	3	2-2′,4′	1848
12	1863	3	2-2′,3′	1864
			3-2′,6′	1866
13	1879	3	4-2′,6′	1878
14	1896	—	4-4′	1894^b
15	1922	4	2-2′,4′,6′	1924^b
16	1935	—	2-3′,5′	1932
			2-2′,3′,6′	1932
			3-2′,5′	1932
17	1943	—	4-2′,[illegible]	1944
18	1952	3	3-2′,4′	1949
19	1963	3	3-2′,3′	1963
			4-2′,4′	1962^b
20	1966	3	3-2′,3′	1963
			4-2′,4′	1962^b
21	1980	3	2-3′,4′	1975
25	2040	4	4-2′,3′,6′	2041
			2,3-2′,4′	2041
26	2051	4	2-2′,3′,4′	2047
			2,3-2′,3′	2055
27	2058	4	2,3-2′,3′	2055
28	2072	4	2,6-3′,4′	2071
30	2097	—	3-2′,3′,5′	2097
			2,4-2′,4′,6′	2097
			2,5-3′,5′	2094
			2,6-2′,3′,5′	2092
31	2115	5	2,3-2′,3′,6′	2120
			2,3-2′,4′,6′	2112
34	2146	4	2-3′,4′,5′	2144
			3-2′,3′,4′	2148
35	2152	— (not 6)	3-2′,3′,4′	2148
			2,4-3′,4′	2154
37	2164	—	2,3-3′,4′	2168
			2,3,6-2′,4′,6′	2160
38	2169	—	2,3-3′,4′	2168
			2,3,6-2′,3′,6′	2173
40	2186	—	2,3-2′,3′,5′	2183
			3,5-2′,3′,6′	2185
46	2246	—	3-2′,4′,5′	2245
			2,4-2′,3′,5′,6′	(2241)
			2,5-2′,3′,4′,6′	(2247)
			2,3,5-2′,3′,6′	2248
47	2254	—	4-3′,4′,5′	2257
			2,3-2′,3′,5′,6′	(2256)
			3,5-2′,3′,5′	2259
			2,4,5-2′,4′,6′	2256

Table II.3.3 (continued)

Peak No.	*R.I.*	*Chloride No.*	*Structure*	*Predicted R.I.*^{c}
49	2283	—	2,3-2',3',4',6'	(2278)
			2,6-2',3',4',5'	(2283)
			3,4-3',4'	2282^{b}
51	2310	5	2,5-3',4',5'	2307
			3,5-2',3',4'	2310
		(7)^{a}	2,3,6-2',3',5',6'	2303
53	2335	5	2,3-3',4',5'	2338
		(7)^{a}	2,3,6-2',3',4',6'	(2329)
54	2340	6	3,5-2',3',5',6'	(2335)
			2,3,5-2',4',5'	2340^{b}
57	2372	—	2,4-2',3',4',5'	(2376)
			2,3,4-2',3',5'	2374
58	2376	6	2,4-2',3',4',5'	(2376)
			3,4-2',3',5',6'	(2379)
			2,3,4-2',3',5'	2374
60	2400	6	3,4-2',3',4',6'	(2401)
			3,4,5-2',3',6'	2396
61	2413	7	2,3,5-2',3',4',6'	(2412)
62	2425	6	2,3,4-2',3',4'	NMR
63	2433	7	2,3,6-2',3',4',5'	(2439)
			2,4,5-2',3',4',6'	(2428)
			2,4,6-2',3',4',5'	(2430)
64	2451	7	2,3,4-2',3',5',6'	(2450)
65	2466	7	2,3,4-2',3',4',6'	(2462)
66	2489	6	2,4,5-3',4',5'	2488^{b}
67	2519	6	3,4-2',3',4',5'	NMR
68	2543	7	2,4,5-2',3',4',5'	(2541)
			3,4,5-2',3',5',6'	(2548)
69	2577	7	2,3,4-2',3',4',5'	NMR

[a] Smaller peaks eluted earlier than major parts of peaks 51 and 53, respectively.
[b] R.I. of synthesised compound.
[c] R.I. between brackets: Predicted R.I. using Table VIII.

Table II.3.4 (from ref. 18)

THE RETENTION INDICES, CHLORINE NUMBERS AND PREDICTED STRUCTURES OF AROCLOR 1242 PEAKS

Peak No.	*R.I.*	*Chlorine No.*^{a}	*Structure*	*Predicted R.I.*^{c}
3	1672	2	2-2'	1669^{b}
6	1765	2	2-3'	1770
8	1781	2	2-4'	1782^{b}
9	1833	3	2-2',5'	1831
10	1849	3	2-2',4'	1848
11	1863	3	2-2',3'	1864
			3-2',6'	1866
12	1879	3	4-2',6'	1878
13	1896	2	4-4'	1894^{b}
15	1935	3	2-3',5'	1932
			3-2',5'	1932
17	1951	3	3-2',4'	1949
18	1961	3	3-2',3'	1963
			4-2',4'	1962^{b}
19	1966	3	3-2',3'	1963
			4-2',4'	1962^{b}
20	1980	3	2-3',4'	1975
21	1996	4	2-2',3',5'	1996
			2,5-2',5'^{a}	1994^{b}
22	2011	4	2-2',4',5'	2012
			2,4-2',5'	2010
24	2024	4	3-2',3',6'	2029
			3-2',4',6'	2021
31	2092	3	4-3',4'	2088
		4	2,5-3',5'	2094
		(5)	2,4-2',4',6'	2097
			2,5-2',3',6'^{a}	2089
34	2138	4	2,5-3',4'^{a}	2138
35	2148	4	2-3',4',5'	2144
			3-2',3',4'	2148

Table II.3.4 (continued): minor peaks in Aroclor 1242

Peak No.	*R.I.*	*Chlorine No.*[d]	*Structure*	*Predicted R.I.*[c]
1	1490	0	Biphenyl	1491[b]
2	1580	—	2-	1577[b]
4	1750	2	2,5-	1744
5	1758	—	2,4-	1756[b]
7	1774	3	2-2',6'	1765
14	1923	4	2-2',4',6'	1924[b]
			2,5-2',6'	1927
16	1944	3	4-2',5'	1944
23	2015	4	2-2',4',5'	2012
25	2028	4	3-2',4',6'	2029
			2,3-2',5'	2025
			2,4-2',4'	2027[b]
			2,6-3',5'	2028
			4-2',4',6'	2033[b]
26	2037	4	4-2',3',6'	2041
27	2041	4	2,3-2',4'	2041
28	2052	4	2-2',3',4'	2047
			2,3-2',3'	2055
29	2061	—	?	
30	2080	3	3-3',4'	2076
32	2121	4	2,3-3',5'	2125
			4-2',4',5'	2125
33	2129	4	2,3-3',5'	2125
			4-2',4',5'	2125
36	2167	4	4-2',3',4'	2160
37	2174	4	2,3-3',4'	2168
38	2178	—	2,4-2',3',5'	2175
			2,5-2',4',5'[a]	2175[b]
			2,6-2',3',4',6'	(2175)
			3,5-2',3',6'	2183
			3,5-2',4',6'	2175
39	2195	5	2,4-2',4',5'[a]	2191
40	2207	5	2,3-2',4',5'	2206[b]
			2,5-2',3',4'[a]	2210
41	2212	5	2,5-2',3',4'[a]	2210
42	2232	5	2,4-2',3',4'	2226
			3,4-2',3',6'[a]	2228
43	2242	6	2,4-2',3',5',6'	(2241)
			2,5-2',3',4',6'	(2247)
			2,3,5-2',4',6'	2240
44	2268	—	2,4-2',3',4',6'	(2263)
			2,3,6-2',4',5'[a]	2264[b]
45	2287	—	2,6-2',3',4',5'	(2283)
			3,4-3',4'	2282[b]
			2,3,4-2',4',6'	2291

[a] Structures found in Aroclor 1254 by NMR determinations.

[b] R.I. of synthesised compound or NMR standard.

[c] R.I. between brackets: Predicted R.I. using Table VIII.

[d] Cl No. between brackets: Chlorine number of minor constituent not associated with accurate R.I.

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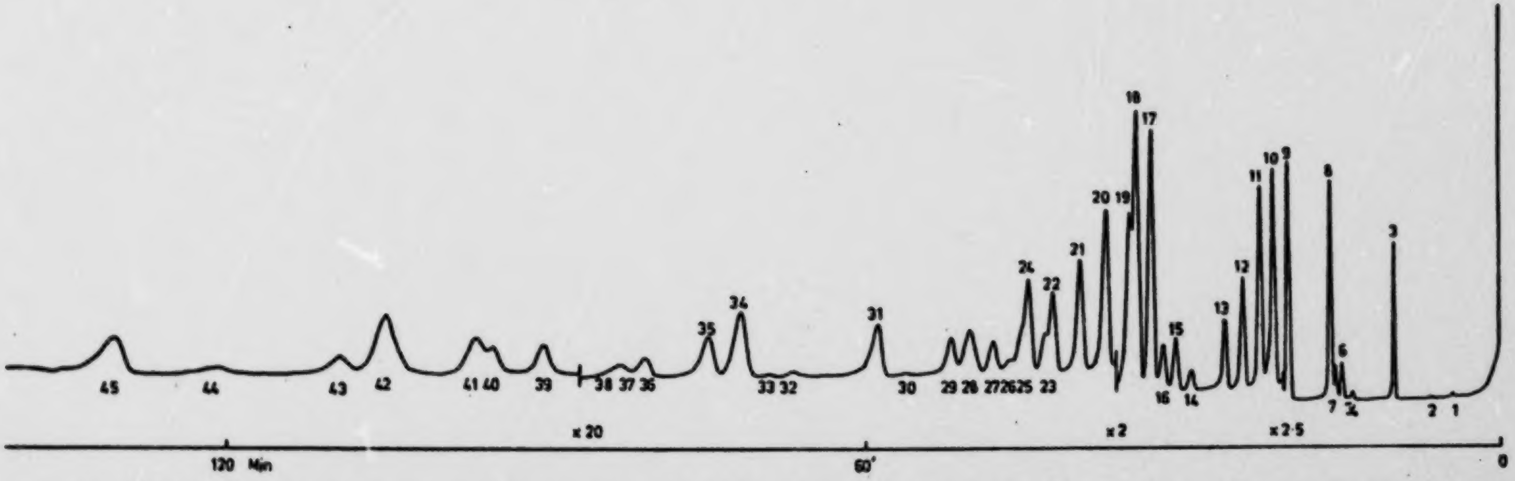
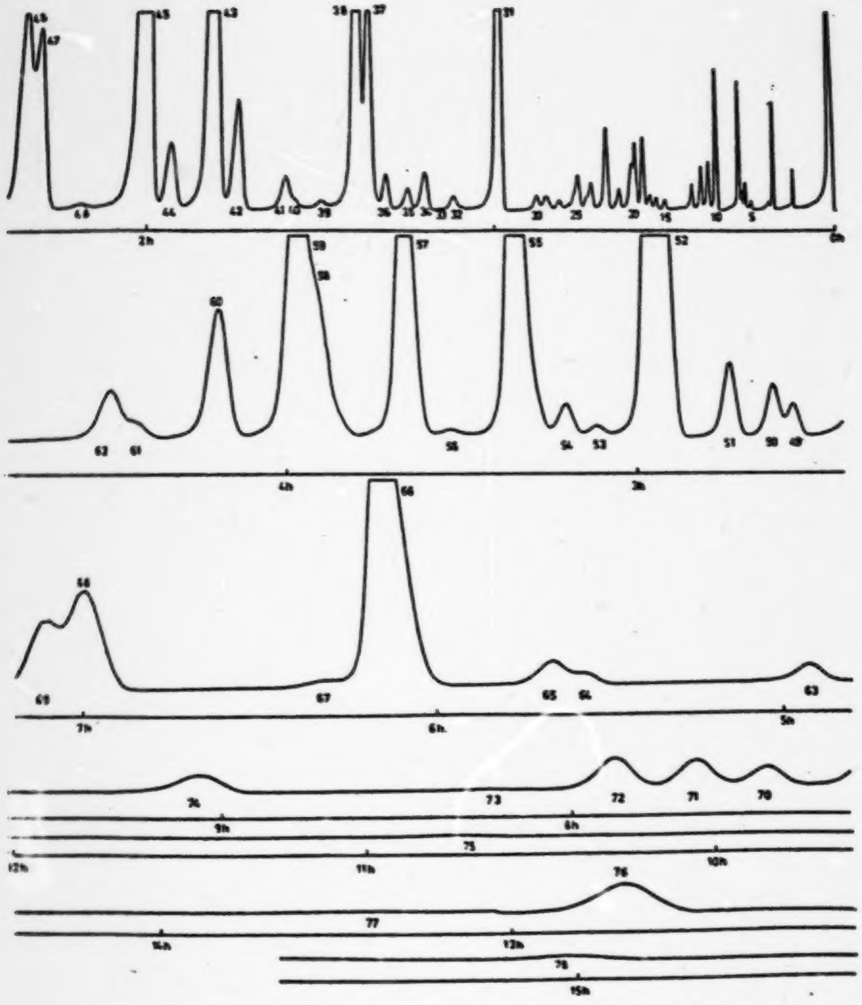


0.2 μl of 1% Aroclor 1242 solution chromatographed on an Apiezon L SCOT column at 205°. Initial attenuation setting of 5 × 10³ reduced as indicated; flame ionisation detector.

Figure II.3.5 (from ref. 18)



0.25 µl 10% Aroclor 1260 solution chromatographed on an Apiezon L SCOT column at 05°. Attenuation setting maintained at 1×10^2; flame ionisation detector.

Figure II.3.6 (from ref. 18)

Table II.3.5 (from ref. 18)

THE RETENTION INDICES, CHLORINE NUMBERS AND PREDICTED STRUCTURES OF AROCLOR 1260 PEAKS

Peak No.	*R.I.*	*Chlorine No.*[a]	*Structure*	*Predicted R.I.*[b]
31	2086	5	2,5-2',3',6'[a]	2089
			2,5-2',4',6'	2081
37	2168	6	2,3,6-2',3',6'	2173
38	2174	5	2,4-2',3',5'	2175
			2,5-2',4',5'[a]	2175[c]
			3,5-2',4',6'	2175
43	2239	6	2,4-2',3',5',6'	(2241)
			2,3,5-2',4',6'	2240
45	2264	6	2,4-2',3',4',6'	(2263)
			2,3,6-2',4',5'[a]	2264[c]
47	2296	6	2,3,4-2',3',6'[a]	2299
48	2300	7	2,3,6-2',3',5',6'	(2303)
			2,4,6-2',3',5',6'	(2295)
52	2358	6	2,5-2',3',4',5'	(2360)
			3,5-2',3',4',6'	(2357)
			2,4,5-2',4',5'[a]	2356[c]
55	2390	6	2,3-2',3',4',5'	(2391)
			2,3,4-2',4',5'[a]	2391[c]
			3,4,5-2',4',6'	2388
		(7)	2,3,5-2',3',5',6'	(2390)
57	2411	7	2,3,5-2',3',4',6'	(2412)
			2,4,5-2',3',5',6'	(2406)
58	2428	6	2,3,4-2',3',4'[a]	2425[c]
		7	2,4,5-2',3',4',6'	(2428)
59	2432	7	2,4,5-2',3',4',6'	(2428)
			2,4,6-2',3',4',5'	(2430)
		8	2,3,5,6-2',3',5',6'	2438[c]
60	2445	7	2,3,4-2',3',5',6'	(2440)
			2,3,6-2',3',4',5'	(2439)
66	2542	7	2,4,5-2',3',4',5'	(2541)
68	2575	7	2,3,4-2',3',4',5'[a]	(2575)
			3,4,5-2',3',4',6'	(2570)
76	2724	8	2,3,4,5-2',3',4',5'	(2726)
Peaks 2–21 in Aroclor 1254 chromatogram were also present in Aroclor 1260.				
22	1994	4	2-2',3',5'	1996
			2,5-2',5'[a]	1994[c]
23	2009	4	2-2',4',5'	2012
			2,4-2',5'	2010
24	2013	—	2-2',4',5'	2012
			2,4-2',5'	2010
25	2022	4	3-2',4',6'	2021
26	2024		2,3-2',5'	2025
			2,4-2',4'	2027[c]
27	2030	—	3-3',5'	2033
			3-2',3',6'	2029
			4-2',4',6'	2033[c]
			2,4-2',4'	2027[c]
			2,6-3',5'	2028
28	2040	4	4-2',3',6'	2041
			2,3-2',4'	2041
29	2050	4	2-2',3',4'	2047
			2,3-2',3'	2055
30	2058	4	2,3-2',3'	2055
32	2117	—	3-2',4',5'	2114
			2,3-2',3',6'	2114

Table II.3.5 (continued): minor peaks in Aroclor 1260

Peak No.	*R.I.*	*Chlorine No.*[c]	*Structure*	*Predicted R.I.*[b]
33	2125	—	4-2′,4′,5′	2125
			2,3-3′,5′	2125
34	2135	4	2,5-3′,4′[a]	2138
35	2146	4	2-3′,4′,5′	2144
			3-2′,3′,4′	2148
36	2158	5	2,5-2′,3′,5′	2159
39	2192	—	2,3-2′,3′,5′	2189
			2,4-2′,4′,5′[a]	2191
40	2205	5	2,3-2′,4′,5′	2206[e]
41	2209		2,5-2′,3′,4′[a]	2210
42	2229	5	2,4-2′,3′,4′	2226
			3,4-2′,3′,6′[a]	2228
44	2254	6	2,3-2′,3′,5′,6′	(2256)
			2,4,5-2′,4′,6′	2256
46	2283	—	2,3-2′,3′,4′,6′	(2278)
			2,6-2′,3′,4′,5′	(2283)
			3,4-3′,4′	2282[e]
49	2321	5	2,4-3′,4′,5′	2323
			3,4-2′,4′,5′[a]	2319
50	2326	7	2,3,6-2′,3′,4′,6′	(2329)
			2,4,6-2′,3′,4′,6′	(2321)
51	2340	6	3,5-2′,3′,5′,6′	(2335)
			2,3,5-2′,4′,5′	2340[e]
53	2372	—	2,4-2′,3′,4′,5′	(2376)
			2,3,4-2′,3′,5′	2374
54	2379	—	2,4-2′,3′,4′,5′	(2376)
			3,4-2′,3′,5′,6′	(2379)
			2,3,4-2′,3′,5′	2374
55	2402	6	3,4-2′,3′,4′,6′	(2401)
61	2460	7	2,3,4-2′,3′,4′,6′	(2462)
62	2464	8	2,3,4,6-2′,3′,5′,6′	(2464)
63	2486	8	2,3,6-2′,3′,4′,5′,6′	(2489)
			2,4,6-2′,3′,4′,5′,6′	(2481)
			2,3,4,6-2′,3′,4′,6′	2490[e]
		6	2,4,5-3′,4′,5′	2488[e]
64	2518	6	3,4-2′,3′,4′,5′[a]	(2516)
65	2522	7	2,3,5-2′,3′,4′,5′	(2525)
67	2550	—	3,4,5-2′,3′,5′,6′	(2548)
69	2578	8	2,3,5-2′,3′,4′,5′,6′	(2577)
			2,3,4,5-2′,3′,5′,6′	(2581)
70	2587	8	2,3,4,5-2′,3′,5′,6′	(2581)
71	2597	8	2,4,5-2′,3′,4′,5′,6′	(2593)
72	2603	8	2,3,4,5-2′,3′,4′,6′	(2603)
73	2618	9	2,3,5,6-2′,3′,4′,5′,6′	(2624)
74	2640	8	2,3,4-2′,3′,4′,5′,6′	(2627)
[d]	—	9	2,3,4,6-2′,3′,4′,5′,6′	(2650)
75	2682	—	3,4,5-2′,3′,4′,5′	(2685)
77	2738	—	3,4,5-2′,3′,4′,5′,6′	(2738)
78	2770	9	2,3,4,5-2′,3′,4′,5′,6′	(2768)
[d]	—	10	2,3,4,5,6-2′,3′,4′,5′,6′	2810[e]

[a] Structures found in Aroclor 1254 by NMR determinations.

[b] R.I. between brackets: Predicted R.I.'s using Table VIII.

[c] Cl No. between brackets: Chlorine number of minor constituent not associated with accurate R.I.

[d] Peaks observed on mass spectrometer oscilloscope, but not on GLC.

[e] R.I. of synthesised compound.

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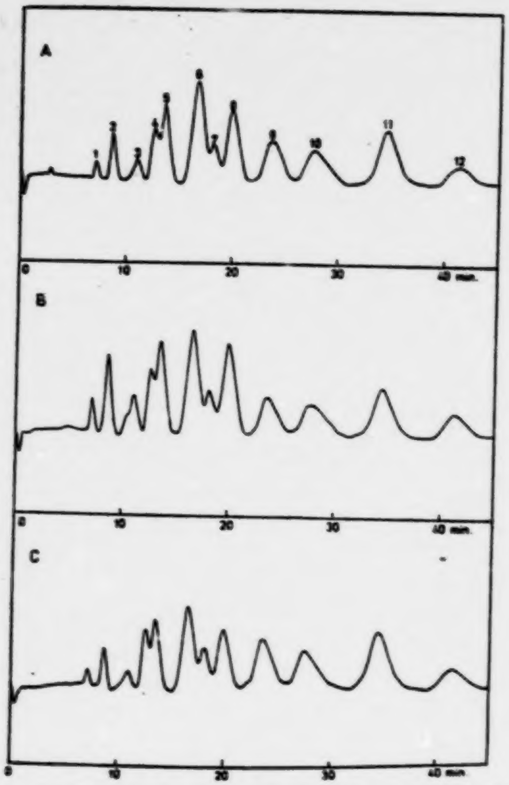
Table II.3.6 (from ref. 27)

TABLE 3. Structural identity of the major PCBs in the gas chromatographic peaks of Aroclors 1254 and 1260

Aroclor 1254 peak No.	Aroclor 1260 peak No.	Predicted chlorine substitutions		Retention time (min)			
				Column 1		Column 2	
		From peak matching	From Table 2	From Fig. 1B,D	Predicted from Table 2	From Fig. 1A,C	Predicted from Table 2
1		[a]2,5,2′,5′		3.35	[e]3.35	2.38	[e]2.38
2		[a]2,5,2′,3′		3.78	3.79	2.90	2.90
3			[d]2,4,2′,3′	4.10	3.88	3.13	2.94
4	1	[a]2,5,2′,3′,6′	[c,d](2,5,3′,4′)	5.01	4.91 (4.97)	3.91	3.84 (3.87)
5	2	[a]2,5,2′ 4′,5′		5.90	5.89	4.43	4.42
6		[a]2,4,2′,4′,5′		—	6.03	4.80	4.48
7a		[a]2,3,2′,4′,5′		—	6.68	5.40	5.38
7b			[c,d]2,5,2′,3′,4′	6.90	6.91	5.70	5.75
8		[a]3,4,2′,3′,6′		7.35	7.28	6.17	6.25
	3	[b]2,3,6,2′,3′,6′	[d](3,4,3′,4′)	7.35	[e]7.20 [e](7.35)	6.38	[e]6.20 [e](6.30)
	4		[d]2,4,2′,3′,4′,6′	8.23	8.17		6.31
9a	5	[a,b]2,4,5,2′,3′,6′		8.79	8.64	7.15	7.13
9b		[b]3,4,2′,4′,5′			8.73		7.19
10	6	[a,b]2,4,5,2′,4′,5′		10.37	[e]10.37	8.17	[e]8.20
11		[c]2,3,4,2′,3′,6′	[c,d](3,4,2′,3′,4′)		10.14 (10.24)	9.30	9.28 (9.36)
	7		[d]2,5,2′,3′,4′,5′	11.27	11.21	9.35	9.33
12	8	[b]2,4,5,2′,3′,4′		12.26	[e]12.16	10.68	[e]10.68
	9		[d]2,4,5,2′,3′,4′,6′	14.12	14.07	11.58	11.56
15			[c,d]2,3,4,2′,3′,4′	14.30	[e]14.27	13.93	[e]13.90
	10	[b]2,3,6,2′,3′,4′,5′		16.28	16.43	14.58	15.06
16				17.15	—	15.93	—
17	11	[b]2,4,5,2′,3′,4′,5′		[f]19.73	—	[f]17.32	—
18	12	[b]2,3,4,2′,3′,4′,5′		23.31	23.13	22.94	22.55

[a] Matched to a peak in Webb and McCall (1972).
[b] Matched to a peak in Tas and Kleipool (1971) or Tas and de Vos (1972).
[c] Indicated to be present by Sissons and Welti (1971).
[d] Substitution giving the closest predicted retention time.
[e] Retention time of standard compound.
[f] Retention time used to calculate $\delta_{2,3,4,5}$.
NOTE: Peak numbering and chromatographic conditions are given under Fig. 1 and in the Materials and Methods section.



Gas chromatograms of *A:* Phenochlor DP 6 (Prodelec, Fr), *B:* Clophen (Bayer, Ger.), *C:* Aroclor 1260 (Monsanto, U.S.)

Column: glass, 1.9 meters × 3 mm i.d. packed with 3% OV-1 on gas-chrom Q, 80–100 mesh, temp 180°C, flow 60 ml N_2/min. Detection: electron capture

Figure II.4.1 (from ref. 22)

Clophen A60 and Clophen A50: these are listed in Table II.4.1 and show good agreement with those for Aroclors 1260 and 1254 respectively (Tables II.3.3, II.3.5).

Six major constituents of Phenochlor DP6 have also been identified (22, 24) as shown in Table II.4.2. These identifications agree with those of the corresponding constituents in Clophen A60 (compounds 11, 19, 29, 32, 38, 49 and 50 in ref. 23). No details appear to have been published of the constitution of the Phenochlor with 42% chlorine which is being imported into the United States (7, p. 210).

Table II.4.3 lists the reported constitution of three commercial Kanechlor mixtures (from refs. 25, 26). Gas-liquid chromatography suggests that the constitution of KC-400 was very similar to that of Aroclor 1248 (Figure II.4.3)*, but the reported correspondence of Kanechlors KC-300 and KC-500 to Aroclors 1242 and 1254 appears to have been less close.

Fenclor DK, now being imported into the United States from Italy at a rate of about 180,000 kg/year (7, p.210), consists of decachlorobiphenyl (ibid.).

II.5 Occurrence of certain substitution patterns in PCB mixtures

The data summarized in Tables II.3.2, II.3.3, II.3.4, II.4.1 and II.4.2 indicate that certain patterns of chlorine substitution are much more frequent than others in commercial mixtures. Figure II.5.1 illustrates the most common substitution patterns on each of the phenyl rings in the biphenyl molecule. By contrast, chlorobiphenyls with substitutions in the 3-; 3,5-; 3,4,5-; and, especially, the 2,4,6- positions are more unusual (18, 23).

Certain substitution patterns are believed to influence the biological activity of chlorobiphenyls (see Section IV below) and it is useful to review the frequency of these substitution patterns in the commercial Aroclor mixtures.

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* See Table II.4.4 for 5 isomers identified in KC-400 (ref. 395).

Table II.4.1 (from ref. 23)

Relative retention times on two GLC columns and amounts of polychlorinated biphenyls in Clophen A 50, A 60 and in human adipose tis

Compound		No. of ortho-chlorines	Relative retention time		Percentage in Clophen		Percentage in Human tissue
No.	Structure[a]		Apiezon L	SF 96	A 50	A 60	
1	2,5-2',5'	2	0.25	0.30	5.0		
2	2,4-2',5'	2	0.26	0.30	1.4		
3	2,3-2',5'	2	0.27	0.33	1.9		1.1
4	(2,-2',3',4')	2	0.30	0.43	1.2		0.66
5	(4-2',3',6')	2	0.32	0.43	2.1		0.56
6	2,5-2',3',6'	3	0.35	0.43	4.4[b]	2.9	1.2
7	2,3-2',3',6'	3	0.38	0.49	2.5[b]	0.28	0.48
8	3,4-2',5'	1	0.41	0.42	3.9		1.5
9		1 or 2	0.42		2.2[c]		2.0[c]
10	2,5-2',3',5'	2	0.45	0.49	2.2	1.1	1.2
11	2,3,6-2',3',6'	4	0.47	0.61	0.50	1.0[d]	
12	2,5-2',4',5'	2	0.48	0.50	7.0[b]	5.6	4.2
13	2,4-2',4',5'	2	0.51	0.51	1.8[b]	[e]	1.9
14	2,3-2',4',5'	2	0.53	0.57	1.4[b]		
15	2,5-2',3',4'	2	0.53	0.58	5.4	1.4	2.3
16	2,3-2',3',4'	2	0.55	0.66	1.0		
17	3,4-2',3',6'	2	0.56	0.62	7.6[b]	2.9	4.7
18	2,3,5-2',3',6'	3	0.60	0.76	1.2	4.2	1.0
19	2,4,5-2',3',6'	3	0.64	0.73	2.0[b]	6.5[d]	0.13
20	2,5-2',3',5',6'	3	0.65	0.68	1.3	3.3	0.43
21	2,3-2',3',5',6'	3	0.70	0.74	0.5		0.05
22	2,3,4-2',3',6'	3	0.72	0.83	1.8	3.2	0.15
23		1 or 2	0.76		0.6[c]		
24	2,3,6-2',3',5',6'	4	0.77	0.91	0.09	0.96	
25	3,4-2',4',5'	1	0.79	0.74	5.0[b]	1.6	5.4
26	2,3,6-2',3',4',6'	4	0.84	0.95	0.05	0.37	
27	2,3,5-2',4',5'	2	0.85	0.86	0.90	2.9	2.7
28	3,4-2',3',4'	1	0.87	0.86	3.6		1.9
29	2,4,5-2',4',5'	2	0.90	0.86	4.2[b]	12.9[d]	21.5
30	2,3,4-2',3',5'	2	0.94	0.97	1.1	1.5	[e]
31		1 or 2	0.96	0.93			
32	2,3,4-2',4',5'	2	1.00	1.00	5.1	11.3[c,d]	14.0
33	2,3,5-2',3',5',6'	3	1.01	1.06	0.04	0.49	0.90
34		4	1.02		0.05[c]		
35		1 or 2	1.04	1.10	1.1[c]	2.0[c]	1.5[c]
36	2,4,5-2',3',5',6'	3	1.08	1.13	0.39	3.3	3.5
37	2,3,4-2',3',4'	2	1.11	1.16	1.3	2.0	0.81
38	2,3,6-2',3',4',5'	3	1.13	1.29	0.33	3.7[d]	[e]
39	2,4,5-2',3',4',6'	3	1.14	1.16	0.17	1.8	2.5
40	2,3,4-2',3',5',6'	3	1.19	1.32	0.27	2.1	1.3
41	2,3,5,6-2',3',5',6'	4	1.23	1.34	0.005	0.07	
42	2,3,4-2',3',4',6'	3	1.28	1.40	0.13	1.3	0.57
43	2,3,4,6-2',3',5',6'	4	1.34	1.44	0.007	0.09	
44	2,4,5-3',4',5'	1	1.41	1.19	0.47	1.0	0.49
45	2,3,6-2',3',4',5',6'	4	1.45	1.59	0.008	0.09	
46	2,3,4,6-2',3',4',6'	4	1.46	1.51	[e]	0.03	
47	3,4-2',3',4',5'	1	1.55	1.37	0.81	1.5	2.0
48	2,3,5-2',3',4',5'	2	1.59	1.49	0.23	0.90	1.2

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Table II.4.1 (continued)

Compound		No. of ortho-chlorines	Relative retention time		Percentage in		
					Clophen		Human tissue
No.	Structure[a]		Apiezon L	SF 96	A 50	A 60	
49	2,4,5-2',3',4',5'	2	1.71	1.56	0.98	7.6[d]	7.7
50	2,3,4-2',3',4',5'	2	1.88	1.82	0.72	4.1[d]	3.9
51	2,3,4,5-2',3',5',6'	3	1.94	1.99	0.08	0.74	0.77
52		1 or 2	2.02	1.96	0.23[c]	1.0[c]	[e]
53	2,3,4,5-2',3',4',6'	3	2.07	2.05	0.06	0.44	0.94
54	2,4,5-2',3',4',5',6'	3	2.15	2.04	0.01	0.28	0.46
55	(2,3,5,6-2',3',4',5',6')	4	2.23				[e]
56	2,3,4-2',3',4',5',6'	3	2.40	2.20	0.01	0.17	0.31
57	2,3,4,6-2',3',4',5',6'	4	2.45	2.56		[e]	[e]
58	3,4,5-2',3',4',5'	1	2.74	2.41	[e]	[e]	
59	2,3,4,5-2',3',4',5'	2	3.18	2.81	0.35	0.67	1.7
60	2,3,4,5,6-2',3',4',5',6'	4	4.12				0.62
					86.7	99.4	100.0 percent
	p,p'-DDE		0.52	0.58			
	p,p'-DDD		0.71	0.73			
	p,p'-DDT		0.90	0.97			

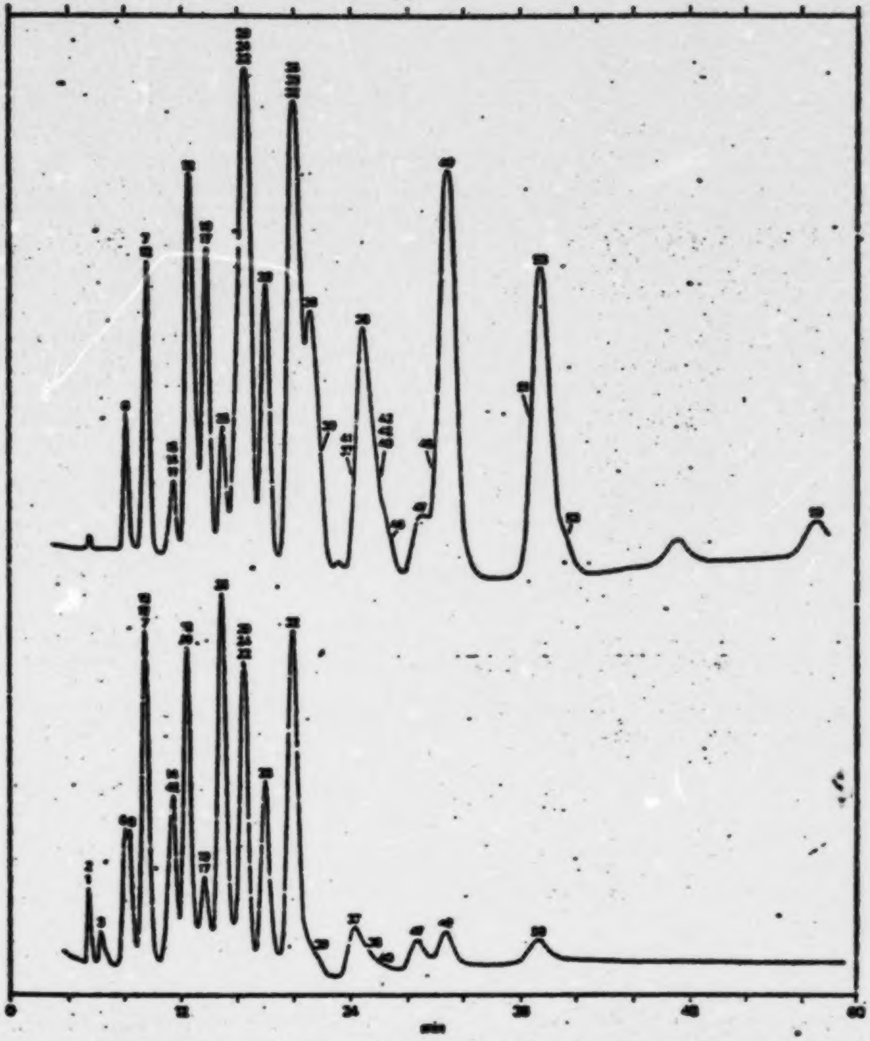
[a] Tentative structures are given in brackets.

[b] Component found present in Aroclor 1254 by Webb and McCall (20)

[c] Figures calculated using responses of chlorobiphenyls with similar retention times in the same fraction from the charcoal column.

[d] Component found present in Phenolclor DP 6 by Tas *et al* (22, 24)

[e] Present in trace amounts only.



Chromatogram of Clophen A 60 (upper trace) and Clophen A 50 (lower trace) on mixed QF 1/SF 96 1.6 m column (2/3 QF 1, 12 percent and 1/3 SF 96, 6 percent) at 200°C.

Only a limited number of figures are shown due to lack of space

Figure II.4.2 Gas-liquid chromatograms of Clophen A60 and Clophen A50, showing numbering of components listed in Table II.4.1.

Table II.4.2 Six constituents identified in Phenochlor DP6 (22, 24)

Peak no. in Figure II.4.1	Identification
3	2,3,6,2',3',6'- hexachlorobiphenyl
5	2,3,6,2',4',5'-hexachlorobiphenyl
6	2,4,5,2',4',5'- hexachlorobiphenyl
8	2,3,4,2',4',5'- hexachlorobiphenyl
10	2,3,4,5,2',3',6'- heptachlorobiphenyl
11	2,3,4,5,2',4',5'- heptachlorobiphenyl
12	2,3,4,5,2',3',4'- heptachlorobiphenyl

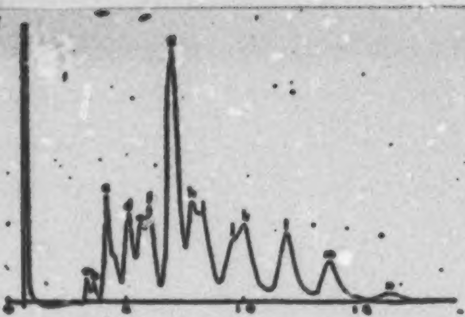


Fig. 1. Kanechlor 400

Table II.4.3 Reported constitution of Kanechlor mixtures

	Kanechlor				
	KC-200	KC-300	KC-400	KC-500	KC-600
Corresponding Aroclor (from ref. 25)	1232	1242	1248	1254	1260
Constitution (from ref. 26)					
2-Cl		17%	3%	-	
3-Cl		60%	33%	5%	
4-Cl		23%	44%	27%	
5-Cl		0.6%	16%	55%	
6-Cl		-	5%	13%	

Table II.4.4. Five constituents identified in Kanechlor KC-400 (ref. 395)

Constituent	Peak no.
2,4,3',4'- tetrachlorobiphenyl	g_1
2,5,3',4'- tetrachlorobiphenyl	g_2
2,3,4,4' - tetrachlorobiphenyl	h
3,4,3',4'- tetrachlorobiphenyl	k
2,3,4,3',4'- pentachlorobiphenyl	m

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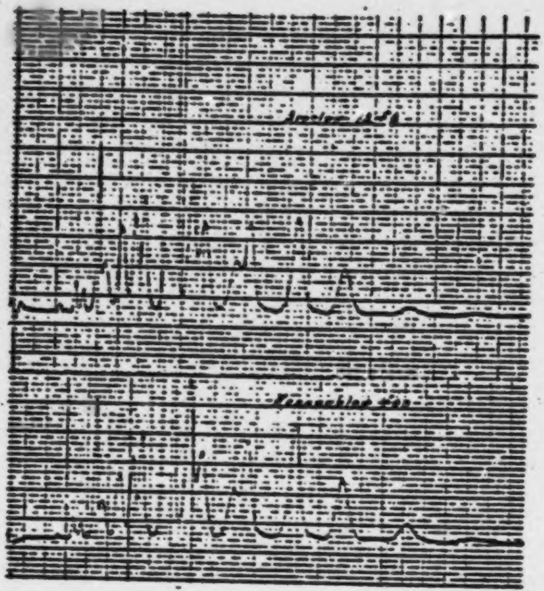
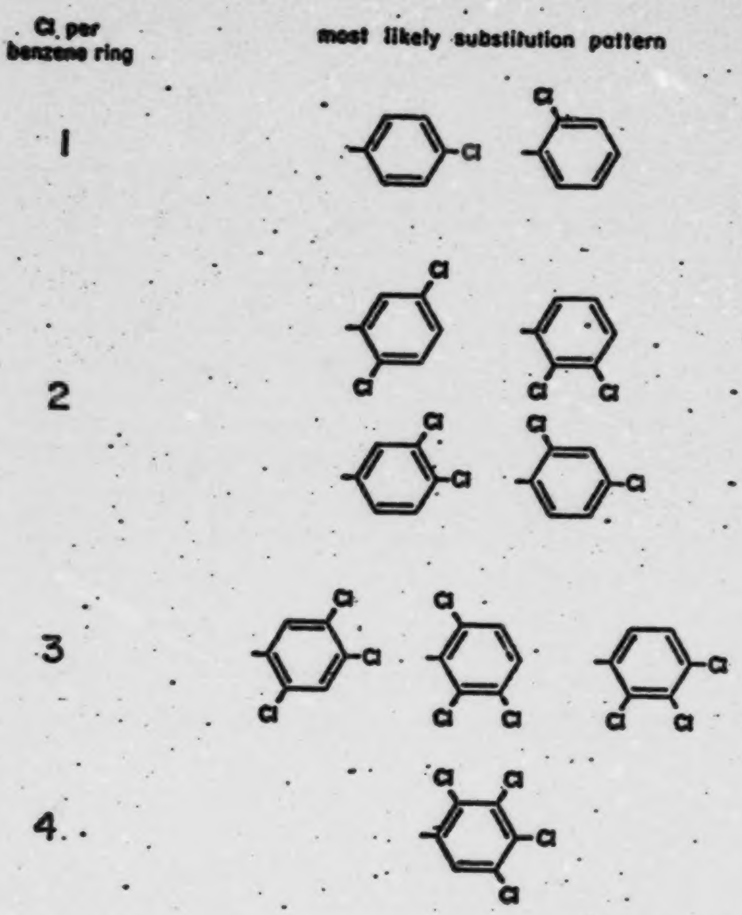


Comparison of gas chromatograms of Kanechlor 400 (Japan) and Aroclor 1248 (USA).

Figure II.4.3 (from ref. 21)

Figure II.5.1
Moieties from Which Principal
Chlorobiphenyl Isomers are Formed



The most common substitution patterns for the chlorobiphenyls found in PCB preparations. Only one phenyl-ring is shown. The most abundant tetrachlorobiphenyls, for example, are those from the dichlorophenyl-moieties shown. One di- and one trichlorophenyl-would give most abundant penta-chlorobiphenyls, etc.

II.5.1 Adjacent unsubstituted positions

The presence of two adjacent carbon atoms without chlorine substitution in one or both rings is believed to facilitate metabolism, because it permits the formation of arene oxide intermediates (28). Essentially all chlorobiphenyls with 5 or fewer chlorine atoms have at least one pair of adjacent unsubstituted carbon atoms, because of the rarity of 3,5- substitution in the natural mixtures (see above). Hence virtually 100 percent of the constituents of Aroclors 1016, 1242 and 1248 fall into this category (Table II.3.1 above). From the data in Table II.4.1 it can be determined that about 92% of the constituents of Clophen A50 and about 66% of the constituents of Clophen A60 have at least one pair of adjacent unsubstituted carbon atoms. The corresponding Aroclors (1254 and 1260) would presumably share these properties.

II.5.2 Number of ortho-chlorines

Jensen and Sundström (23) presented evidence that chlorobiphenyls with three or four chlorine atoms in the ortho-positions (2- and 6- positions) are more easily metabolized than those with only one or two ortho-chlorines. Table II.5.1 shows that compounds with three or four chlorines in the ortho-position are virtually absent from Aroclor 1242 (and from Aroclor 1016), but are fairly well represented in Clophens A50 and A60 (which correspond to Aroclors 1254 and 1260).

II.5.3 4,4'-substitution

McKinney (29) has suggested that chlorobiphenyl isomers with chlorine substitution in both the 4- and 4'- positions tend to be biologically active and well retained in tissues. Table II.5.2 shows that the number and proportion of these isomers increases with increasing chlorination of the mixture.

Table II.5.1 Frequency of compounds with various numbers of ortho-chlorine substitutions and with 4-4' substitution in selected PCB mixtures.

	Aroclor 1242 (from Table II.3.4)		Clophen A50 (from ref. 23)		Clophen A60 (from ref. 23)	
	percent of total	no. of compounds	percent of total	no. of compounds	percent of total	no. of compounds
4- ortho-chlorines	0	0	0.7	8	2.6	9
3 ortho-chlorines	<1	4	15.2	17	34.7	16
2 or 1 ortho-chlorines	99	45	69.8	32	62.0	20
4-4' substitution	10-15	8	25.0	18	47.7	18
3-4 substitution						
on one ring	ca.20	17	35.4	21	38.2	19
on both rings	<1	1	23.0	17	49.6	18

Note: information for Aroclor 1242 is less complete and precise because the compounds in this mixture were not all identified unequivocally (18).

II.5.4. 3,4 - substitution

McKinney et al. (30) have also shown an association between biological activity and substitutions in the 3,4-, 4,5-, or 3,4,5- substitutions on one or both rings. 3,4- and 4,5- substitutions are frequently found in PCB mixtures, but 3,4,5- is usually found only as part of a 3,4,5,6- substitution pattern (Figure II.5.1). Table II.5.1 shows that chlorobiphenyl isomers with 3,4- or 4,5- substitutions are frequent in PCB mixtures and constitute more than half of the components in the more chlorinated mixtures. However, 3,4,5,3',4',5'- hexachlorobiphenyl, an exceptionally toxic isomer (30), has not been detected in commercial mixtures. The nearest equivalent, 3,4,5,2',3',4',5'- heptachlorobiphenyl, is present only in trace quantities (Tables II.3.5, II.4.1), and 2,3,4,5,2',3',4',5'- octachlorobiphenyl is only a minor constituent (ibid.). 3,4,3',4'- tetrachlorobiphenyl, which has an analogous structure, is probably a minor constituent of Aroclor 1242, but may be absent from Aroclor 1016 (Table II.3.4., Figure II.3.3)*.

II.5.5 Toxic contaminants in commercial PCB mixtures

Vos et al. (31) isolated a highly toxic fraction from samples of Clophen A60 and Phenoclor DP-6 and tentatively identified in it small quantities of polychlorinated dibenzofurans (PCDFs). This identification has been confirmed by Bowes et al. (32), who found several chlorodibenzofurans (CDFs) with 4-6 chlorine atoms per molecule in the same mixtures. The total quantity of PCDFs in Clophen A-60 and Phenoclor DP-6 was estimated to be 8.4 and 13.6 ppm respectively (Table II.6.1). PCDFs were also detected in American-manufactured Aroclors 1248, 1254 and 1260, at total concentrations varying from 0.8 ppm (Aroclor 1260) to 2.0 ppm (Aroclor 1248) (Table II.5.2). PCDFs were not detected in a single sample of Aroclor 1016 (32).

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* This isomer is a major constituent of KC-400 (Table II.4.4).

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Table II.5.2 (from ref. 32)

Chlorinated dibenzofuran concentrations* in Aroclor, Clophen and Phenoclor

PCB	4-Cl	5-Cl	6-Cl	Total
Aroclor 1248 (1969)	0.5 (25)	1.2 (60)	0.3 (15)	2.0
Aroclor 1254 (1969)	0.1 (6)	0.2 (12)	1.4 (82)	1.7
Aroclor 1254 (1970)	0.2 (13)	0.4 (27)	0.9 (60)	1.5
Aroclor 1260 (1969)	0.1 (10)	0.4 (40)	0.5 (50)	1.0
Aroclor 1260 (lot. AK3)	0.2 (25)	0.3 (38)	0.3 (38)	0.8
Aroclor 1016 (1972)	ND	ND	ND	—
Clophen A-60	1.4 (17)	5.0 (59)	2.2 (26)	8.4
Phenoclor DP-6	0.7 (5)	10.0 (74)	2.9 (21)	13.6

*Expressed as μg g^{-1} PCB. Values in parentheses represent quantity as percentage total dibenzofuran.

ND, not detected (<0.001 μg g^{-1}).

Amounts of PCB ranging from 1.0 to 2.0 g were dissolved in 400 ml hexane, placed on a Florisil column (180 g, internal diameter 31.5 mm), and eluted with: an additional 1,600 ml hexane, and successive 800 ml volumes each of 5% diethyl-ether–hexane, 25% diethyl-ether–hexane and acetone, at a rate of approximately 7 ml min^{-1}. The major portion of the PCB was eluted in the hexane fraction, which was discarded. On addition of the 5% mixture, the eluates were collected in six successive 400 ml volumes. To eliminate the polar solvents, each eluate was evaporated twice just to dryness and taken up each time in a minimal amount of hexane. Each fraction, in 1 ml hexane, was placed on a microalumina column[14] and eluted with 10 ml each of 1% and 20% methylene chloride in hexane. These were also taken twice just to dryness and made to up a volume of 1 ml in hexane to eliminate the methylene chloride before gas chromatographic analysis. Aliquots of all fractions obtained before and after partitioning on alumina were injected into a six foot glass column containing 3% OV1 on 100–120 mesh Supelcoport in Tracor MT220 and Hewlett-Packard 5700 gas chromatographs equipped with ^{63}Ni electron-capture detectors. PCBs were found to be present in each fraction eluted from the Florisil column in amounts sufficient to interfere with the detection of trace contaminants. Partitioning on the alumina columns separated most of the PCB interference into the 1% methylene chloride fractions. On removal of this interference, different peak patterns appeared in the chromatograms of the 20% methylene chloride fractions. Compounds eluting in the 20% methylene chloride fraction were collected for mass spectrometric analysis using a 20:1 effluent splitter, and a trap consisting of a capillary tube (1 mm internal diameter, 100 mm long) bent to a U shape, immersed in a liquid nitrogen bath. Methylene chloride (20%; 4 μl) in hexane was injected into the capillary as a rinse, removed with a 1.0 μl micropipette, and placed directly on the mass spectrometer probe. The probe was inserted into a GEC AEI MS902 high resolution mass spectrometer and the solvent removed by the force pump. The probe was rapidly inserted into the ion source and multiple scans were recorded in the on-line high resolution mode[15].

PCDFs have also been detected in several Japanese-made PCBs at concentrations of 1-33 ppm (33-35), with the highest concentration in a sample of KC-400 (35) (Table II.5.3).

The CDFs present in commercial PCB mixtures range from dichloro- to heptachlorodibenzofurans (33, 34), but most of those detected have been tetrachloro-, pentachloro- and hexachloro-dibenzofurans (32-34). At least 11 CDFs were detected in the European and American products (32) and at least 15 in KC-400 (35). The only isomers that have been precisely identified to date are 2,3,7,8- tetrachlorodibenzofuran and 2,3,4,7,8- pentachlorodibenzofuran (36). (There are 119 structurally different CDF isomers.)

Polychlorinated napthalenes (PCNs) have also been identified in small quantities in Clophen A60, Phenoclor DP-6, Aroclor 1254 and KC-400 (31-33).

Despite occasional statements that commercial PCBs may contain polychlorinated dibenzo-p-dioxins (PCDDs), there appear to be no authenticated reports of their presence (32). The structure of PCDFs and PCDDs is shown in Figure II.5.2.

II.6 Physical properties of chlorobiphenyls and PCB mixtures.

II.6.1 General properties

The general physical properties of some representative chlorobiphenyls are summarised in Table II.6.1; those of the Aroclor mixtures, including Aroclor 1016 and Aroclor 1270 (decachlorobiphenyl), are summarised in Table II.6.2. Although most chlorobiphenyls are solids at room temperature, lower chlorinated mixtures (e.g., Aroclors 1221, 1232, 1242, and 1248) are mobile oils and increasing chlorine content results in mixtures taking on the consistency of viscous liquids (Aroclor 1254) or sticky resins (Aroclor 1260

Table II.5.3 (from ref. 34)

Concentration of chlorinated dibenzofurans in Kanechlors and "Yusho oil".

Samples		Chlorodibenzofurans						Concentration (ppm)	
		Di-	Tri-	Tetra-	Penta-	Hexa-	Hepta-	a	b
Kanechlors	300			+	+			1	1.5
	400	+++	+++ ++	+++ ++	+++			18	17
	500				+	+++	+	4	2.5
	600			+	+	++	++	5	3
"Yusho oil"	A		+	++	+++	+		5	4.5
	B		+	++	++	+		4	5
	C		+	++	+++	+		5	5

a: Calculated from peak heights, b: Calculated by perchlorination method.

Figure II.5.2.

Skeletal structures of: *a*, chlorinated biphenyl, $x+y$ = 1–10; *b*, chlorinated dibenzofurans, $x+y$ = 1–8; *c*, chlorinated dibenzodioxins, $x+y$ = 1–8.

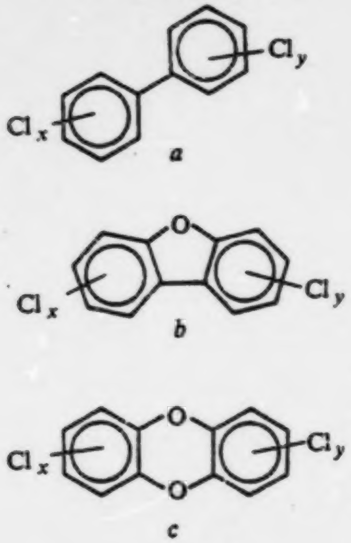


Table II.6.1 (from ref. 21)

PHYSICAL PROPERTIES OF CHLOROBIPHENYLS (Hubbard, 1964; Cook, 1972)

Compound	Melting point, °C	Boiling point, °C (mm Hg)
*2-chloro-	34	267–268; 154(12)
3-chloro-	89	284–285
*4-chloro-	76	125(14)
*2,2′-dichloro-	61	
3,3′-dichloro-	29	322–324; 320–326
*4,4′-dichloro-	148	315–319
3,5-dichloro-	36	166(10)
2,5-dichloro-		171(15); 182(30)
3,4-dichloro-	49	195–200(15)
2,3-dichloro-		172(30)
*2,4′-dichloro-	44	
3,4,3′,4′-tetrachloro-	172	230(50)
3,4,2′,5′-tetrachloro-	103	
2,6,2′,6′-tetrachloro-	198	
*2,5,3′,5′-tetrachloro-	162	
2,4,2′,4′-tetrachloro-	83	
*2,5,2′,5′-tetrachloro-	85	
2,4,5,3′,4′-pentachloro-	179	195–220(10)
2,3,4,5,2′,4′,5′-heptachloro-		240–280(20)

* Present in commercial mixtures.

Table II.6.2 (from ref. 7)

NR = not reported
* = same chlorine content as does chlorobiphenyl
** = polychlorinated terphenyls

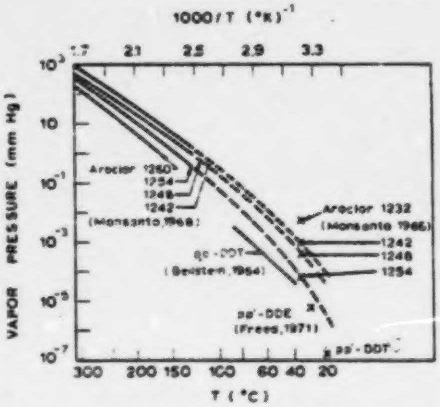
Chemical and Physical Properties of Representative Aroclors

Property	Aroclor 1221	Aroclor 1232	Aroclor 1016	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260	Aroclor 1262	Aroclor 1268	Aroclor 1270*	Aroclor 5442	Aroclor 5460**
Appearance	Clear, mobile oil	Clear, mobile oil	Clear, mobile oil	Clear, mobile oil	Clear, mobile oil	Light-yellow viscous liquid	Light-yellow soft, sticky resin	Light-yellow sticky viscous resin	White to off-white powder	White crystalline powder	Yellow, transparent sticky resin	Clear yellow-to-amber brittle resin
Color, maximum	100 APHA	100 APHA		100 APHA	100 APHA	100 APHA	150 APHA	150 APHA	1.5 NPA (molten)			
Chlorine, percent	20.5-21.5	31.4-32.5	~41	42	48	54	60	61.5-62.5	68	71	42	60
Acidity, mg KOH/g maximum	0.014	0.014		0.015	0.010	0.010	0.014	0.014	0.05			
Moisture, ppm, maximum	-	-		50	50	50	50	-	-			
Ave. coefficient of expansion, cc/cc/°C	0.00071 (15°-65°C)	0.00073 (25°-100°C)		0.00068 (25°-65°C)	0.00070 (25°-65°C)	0.00066 (25°-65°C)	0.00067 (20°-100°C)	0.00064 (25°-65°C)	0.00067 (20°-100°C)			
Specific gravity	1.182-1.192 (25°/15.5°C)	1.270-1.280 (25°/15.5°C)	1.362-1.372 (25°/15.5°C)	1.381-1.392 (25°/15.5°C)	1.405-1.415 (65°/15.5°C)	1.495-1.505 (65°/15.5°C)	1.555-1.566 (90°/15.5°C)	1.572-1.583 (90°/15.5°C)	1.804-1.811 (25°/25°C)	1.944-1.960 (25°/25°C)	1.470 (25°/25°)	1.670 (25°/25°)
Density, lb/gal, 25°C	9.85	10.55	11.40	11.50	12.04	12.82	13.50	13.72	15.00	16.30	12.25	13.90
Distillation range, °C, corrected (ASTM D-20, modified)	275-320	290-325	323-356	325-366	340-375	365-390	385-420	390-425	435-450	450-460	215-300	280-335
Evaporation loss, %, 100°C, 6 hr	1.0-1.5	1.0-1.5		0-0.4	0-0.3	0-0.2	0-0.1	0-0.1	0-0.06			
(ASTM D-6, mod.) 163°C, 5 hr				3.0-3.6	3.0-4.0	1.1-1.3	0.5-0.8	0.5-0.6	0.1-0.2			
Flash point °C (Cleveland Open °F Cup)	141-150 286-302	152-154 305-310	170	176-180 348-356	193-196 379-384	none to boiling point	none to boiling point	none to boiling point	none to boiling point	none	247	none
Fire point °C (Cleveland Open °F) Cup)	176 349	238 460	none to boiling point	none to boiling point	none to boiling point	none to boiling point	none to boiling point	none to boiling point	none to boiling point	none	>350	none
Pour point °C (ASTM D-97) °F	1 (crystals) 34 (crystals)	-35.5 -32		-19 -2	-7 19.4	10 50	31 88	35-38 95	-		46	—
Softening point °C (ASTM E-28) °F	-	-		-	-	-	-	-	150-170 302-338 (ball point)	245-300	46-52	98-105.5
Refractive index, n D-20 20°C	1.617-1.618	1.620-1.622	1.622-1.624 (@ 25°C)	1.627-1.629	1.630-1.631	1.639-1.641	1.647-1.649	1.6501-1.6517	-			1.660-1.665
Viscosity, sec Saybolt Universal (ASTM D-88) 100°F (37.8°C) 130°F (54.5°C) 210°F (98.9°C)	38-41 35-37 30-31	44-51 39-41 31-32	71-81 NR NR	82-92 49-56 34-35	185-240 73-80 36-37	1800-2500 260-340 44-48	3200-4500 72-78	600-850 (160°F; 71°C) 86-100	-			
Average No. of Cl/mol	1.15	2.04	NR	3.10	3.90	4.96	6.30	6.80	8.70	NR	NR	NR

and 1262). This results from the mutual depression of melting points of the components. Resinous Aroclors retain their strictly resinous state permanently; they do not "dry" even when exposed to air in thin films. With the exception of Aroclor 1221 and 1268, PCBs and Aroclors do not crystallize upon heating or cooling but at a specific temperature, defined as a "pour point", change into a resinous state.

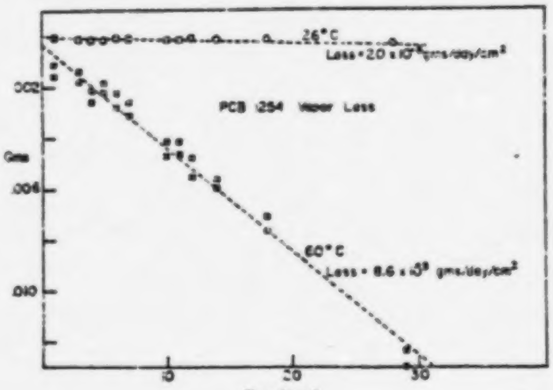
II.6.2 Vapor pressure and volatilization

Figure II.6.1 summarizes the vapor pressures of four Aroclor mixtures as functions of temperature (2). Figure II.6.2 displays the results of measurements of the vapor loss of Aroclor 1254 from its own surface at two temperatures (37). Table II.6.3 compares the vaporization rates of a range of Aroclor mixtures from their own surfaces at the same temperature (4). Both vapor pressure and vaporization rate are much higher in the less chlorinated mixtures; this indicates that the lower chlorinated chlorobiphenyls are much more volatile than the higher chlorobiphenyls. A consequence of this difference in volatility is that the mixtures are differentiated on vaporization. Figure II.6.3. shows chromatograms of Aroclor 1016 and of vapors volatilizing from it (13): the components with shortest retention times (dichlorobiphenyls -- see Figure II.3.3) are much more prominent in the vapor than in the standard, whereas the trichlorobiphenyls are much less volatile and the tetrachlorobiphenyls are hardly represented at all in the vapors. Similar differentiation of the chlorobiphenyls with 4-7 chlorine atoms is illustrated in Figure II.6.4, which shows measurements of vapor loss of components of Aroclor 1254 from dry sand and cycled sand (alternately moistened with water and dried) (37).



Vapor pressures of selected Aroclors, *p,p'*-DDT, and *p,p'*-DDE.

Figure II.6.1 (from ref. 2)



Loss of Aroclor 1254 from itself as a function of time

Figure II.6.2 (from ref. 37)

Table II.6.3 (from ref. 4)

VAPORIZATION RATES OF AROCLORS

Aroclor (surface area: 12.28 cm^2)	Wt. loss (g)	Exposure at 100°C (hr)	Vaporization rate (g/cm^2/hr)
1221	0.5125	24	0.00174
1232	0.2572	24	0.000874
1242	0.0995	24	0.000338
1248	0.0448	24	0.000152
1254	0.0156	24	0.000053
1262	0.0039	24	0.000013
1260	0.0026	24	0.000009
4465	0.0064	72	0.000007
5442	0.0039	72	0.000004
5460	0.0032	72	0.000004

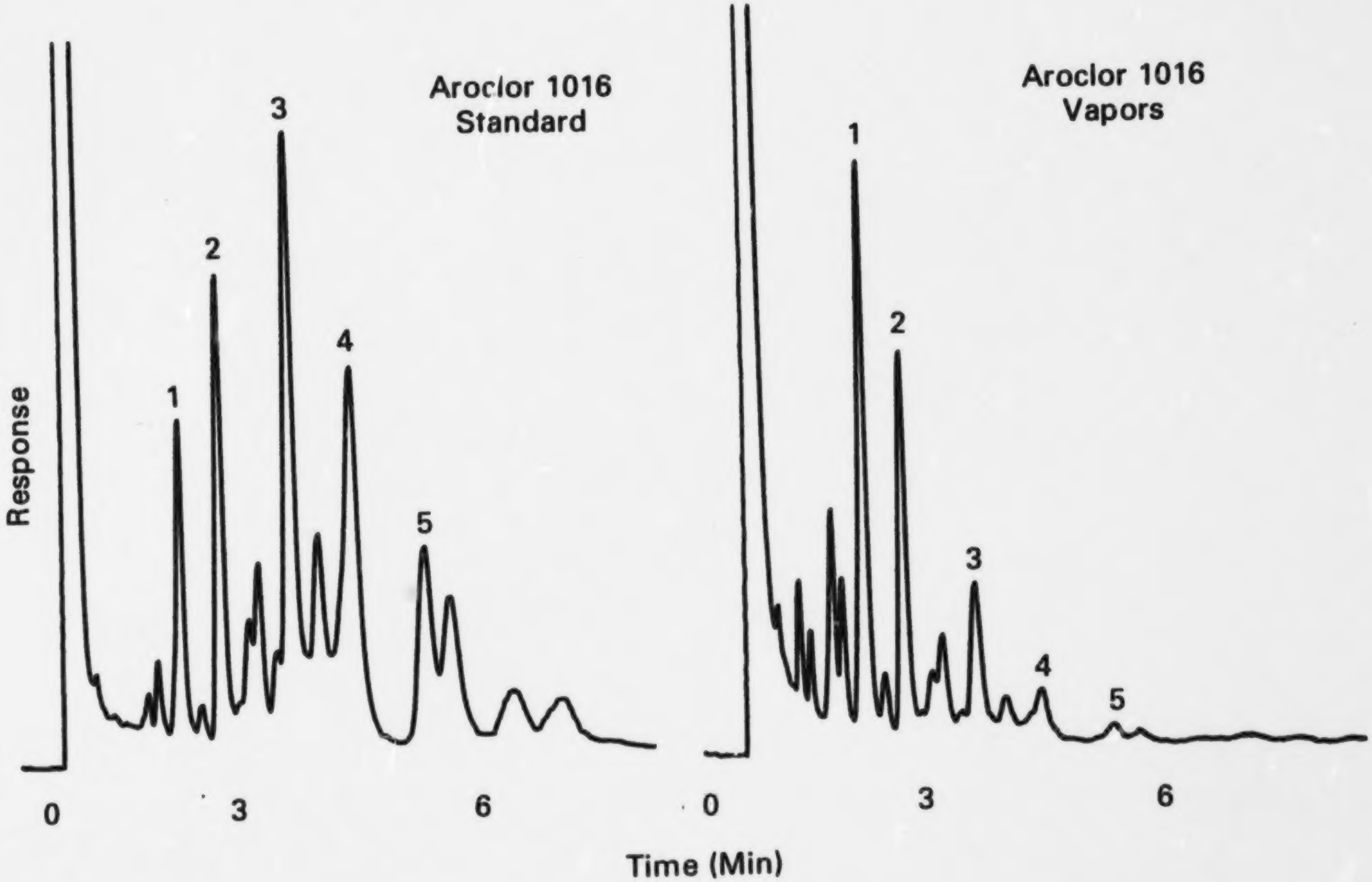
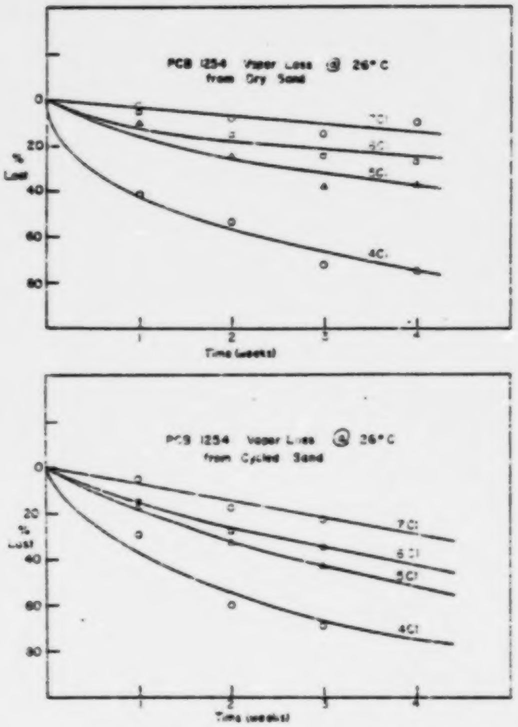


Figure II.6.3. Gas-liquid chromatograms illustrating differential volatilization of components of Aroclor 1016 (13).



Loss of Aroclor 1254 from an Ottawa sand

Figure II.6.4 (from ref. 37)

II.6.2.1 Factors affecting the rate of volatilization

Figure II.6.4 shows that the rate of evaporation of the higher chlorobiphenyls (7, 6 and 5 chlorine atoms) from sand was markedly increased by cycling with water, but that the volatilization of tetrachlorobiphenyls was not affected much. In other words, the periodic evaporation of water from the sand enhanced the total volatilization of PCBs, but reduced the degree of differentiation of the chlorobiphenyls. When heated at 100°C in water solution (conditions of co-distillation), however, the total volatilization of PCBs was reduced, and the degree of differentiation was increased (Table II.6.4) (4, 38). It is known from studies of pesticides that soil moisture and evaporation of water have a strong influence on the rate of volatilization of chlorinated hydrocarbons from soils and sand.

MacKay and Wolkoff (39) calculated theoretical evaporation rates for various Aroclors from water, assuming that they are in true solution, that the solution is well mixed, and that the vapor formed is in equilibrium with the liquid at the interface. Because of the very high activity coefficients of chlorobiphenyls in water, this analysis predicted very rapid volatilization rates (Table II.6.5). In fact, under laboratory conditions, PCBs appear to volatilize fairly rapidly from water in aquaria (40) and even from flasks plugged with glass wool (41). In the latter experiment, the time required for half of a 25 µg/l solution of Aroclor 1260 to disappear was of the order of 6-12 weeks; only a small fraction was recovered from the glass wool. However, in the same conditions volatilization was markedly reduced in the presence of sediments (42), and there was then little or no volatilization between 6 and 12 weeks. Hence, in natural waters, it seems likely that adsorption to sediments (see below) would limit the rate of vapor loss to the atmosphere.

Table II.6.4 (from ref. 32)

PERCENT LOSS IN AREA OF SEVEN CHROMATOGRAM PEAKS OF AROCLOR AFTER HEATING

Aroclor 1254 peak	% Peak Remaining After Heating with water 25 min	 60 min	 without water 10 min
1	34	17	13
2	59	26	15
3	78	27	20
4	60	46	20
5	86	49	27
6	100	85	28
7	100	67	16

Table II.6.5 (from ref. 39)

Solubility, Vapor Presure and Halflife for Vaporization from Water of Selected Aroclors at 25°C

PCB Type	Solubility (mg/1)	Vapor Pressure (mm Hg)	Theoretical halflife for vaporization from 1 m. water column
Aroclor 1242	0.24	4.06×10^{-4}	5.96 hr.
Aroclor 1248	5.4×10^{-2}	4.94×10^{-4}	58.3 min.
Aroclor 1254	1.2×10^{-2}	7.71×10^{-5}	1.2 min.
Aroclor 1260	2.7×10^{-3}	4.05×10^{-5}	28.8 min.

II.6.3 Solubility in water

Table II.6.6 summarizes two sets of measurements of the solubility of individual chlorobiphenyls in water (43, 44). For the four chlorobiphenyls studied in both experiments, the results of Haque and Schmedding (44) indicate much lower solubilities than those of ref. 43. Metcalf et al. (45) give still lower values for the water solubilities of two of the same chlorobiphenyl isomers (0.016 ppm for 2,5,2'- trichlorobiphenyl and 0.016 ppm 2,5,2',5'- tetrachlorobiphenyl). Haque and Schmedding (44) attributed the discrepancy to the long period required to achieve equilibrium between the chlorobiphenyls and water: they allowed more than one month to achieve equilibrium, whereas Wollnöfer et al. (43) allowed only two hours. However, Schoor (46), in a very detailed study, has given evidence that "solutions" of chlorobiphenyls in water are in fact stable emulsions of chlorobiphenyl aggregates. A "conservatively high" estimate based on ultracentrifugation experiments indicated that the average solubility of the constituents of Aroclor 1254 is less than 0.1 μg/l (0.1 parts per billion) in fresh water and less than 0.04 μg/l in salt water (46).

Although the physical nature of the "solutions" of chlorobiphenyls and their absolute solubilities in water are thus in serious question, there is no doubt that the equilibrium concentrations of the "solutions" are much higher for the lower chlorinated biphenyls (Table II.6.6). This is reflected in the reported "solubilities" of the commercial mixtures: over 200 ppb for Aroclor 1221, 225-250 ppb for Aroclor 1016, 200 ppb for Aroclor 1242, 48-100 ppb for Aroclor 1248, 40 ppb for Aroclor 1254, and 25 ppb for Aroclor 1260 (2, 4, 13, 47). It is also reflected in differential uptake of the various components of the mixtures into "solution". Figure II.6.5, for

Table II.6.6. Solubility in water of various chlorobiphenyl isomers as reported by two groups of authors (43, 44)

	Solubility (ppm)	
Compound	(Wollnöfer *et al.*, 1973)	(Haque and Schmedding, 1975)
Monochlorobiphenyls		
2-	5.9	
3-	3.5	
4-	1.19	
Dichlorobiphenyls		
2,4-	1.40	
2,2'-	1.50	
2,4'-	1.88	0.637 ± 0.004
4,4'-	0.08	
Trichlorobiphenyls		
2,4,4'-	0.085	
2',3,4-	0.078	
2,2',5-	—	0.248 ± 0.004
Tetrachlorobiphenyls		
2,2',5,5'-	0.046	0.0265 ± 0.008
2,2',3,3'-	0.034	
2,2',3,5'-	0.170	
2,2',4,4'-	0.068	
2,3',4,4'-	0.058	
2,3',4',5-	0.041	
3,3',4,4'-	0.175	
Pentachlorobiphenyls		
2,2',3,4,5'-	0.022	
2,2',4,5,5'-	0.031	
Hexachlorobiphenyl		
2,2',4,4',5,5'-	0.0088	0.000953 ± 0.00001
Octachlorobiphenyl		
2,2',3,3',4,4',5,5'-	0.0070	
Decachlorobiphenyl	0.015	

AROCLOR 1016

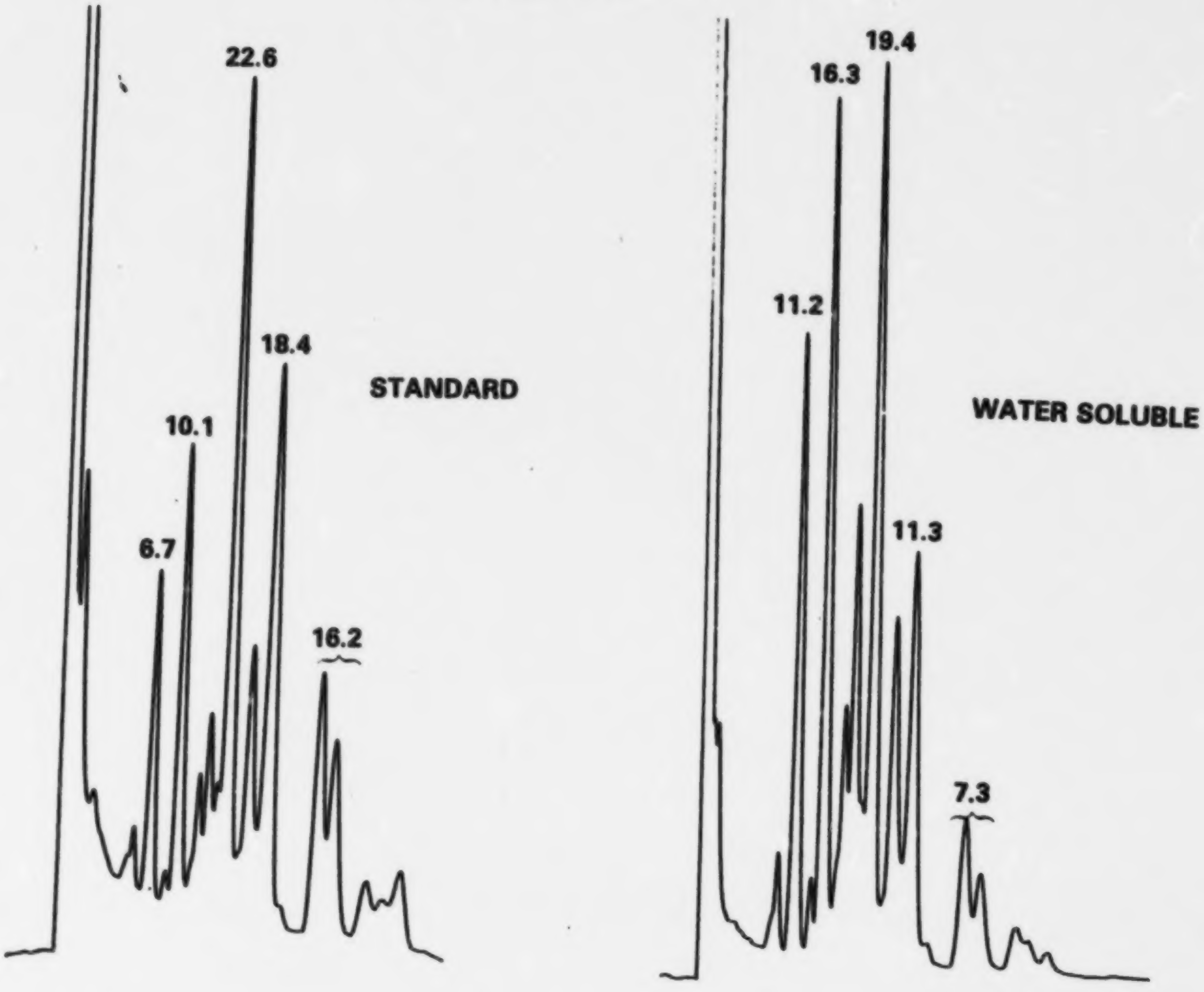


Figure II.6.5 (from ref. 13)

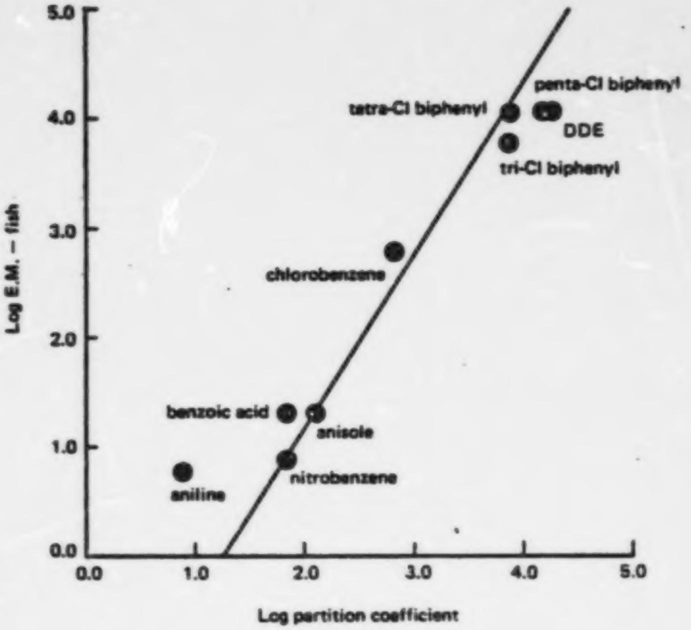
example, compares a gas-liquid chromatogram of Aroclor 1016 with that of an extract from a water "solution": it shows that the dichlorobiphenyls are much better dispersed in water than the tri- and tetra-chlorobiphenyls. Similar differentiation of the components of Aroclor 1254 on dispersal into water has been demonstrated (2, 4, 37, 38).

II.6.4 Solubility in organic solvents and partitioning into lipids

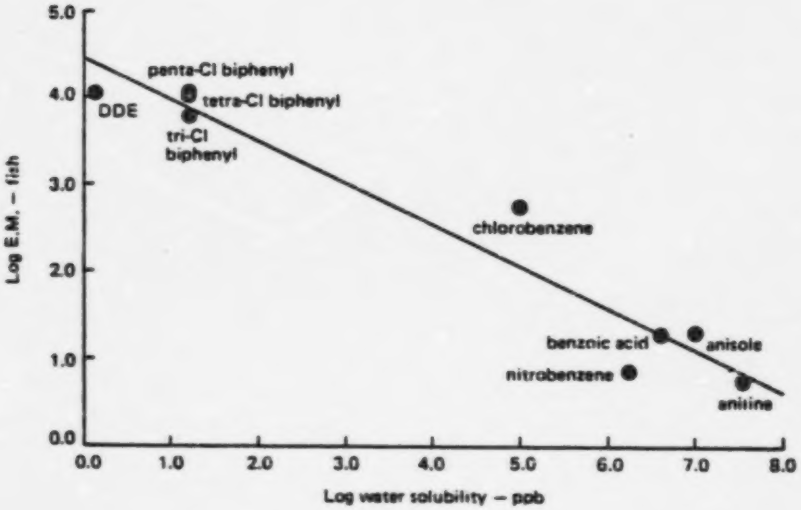
Chlorobiphenyls are freely soluble in many organic solvents (4, Table 4). As a consequence of their very low solubilities in water (Table II.6.6) they are very strongly partitioned into organic solvents, including lipids in biological systems (2, 45). Metcalf et al. (45) give figures in the range 10-20,000 for the partition coefficients between octanol and water of representative tri-, tetra-, and pentachlorobiphenyls. Partition coefficients between these two solvents have been found to correlate well with ecological magnification factors in aquatic organisms (45 - see Figure II.6.6).

II.6.5 Adsorption to soils, sediments, and particulates

PCBs are strongly adsorbed from water onto solid surfaces, including glass and metal surfaces in laboratory apparatus (46) and soils, sediments and particulates in the environment (37, 42, 48-51). Figure II.6.7 illustrates the adsorption of Aroclor 1254 from a water solution onto a variety of particulate materials (37). Adsorption was much more efficient onto Woodburn soil (with a high organic content) and clays than onto sand or silica gel. Another study (47) showed that Aroclor 1016 was extremely strongly adsorbed to a silty clay loam soil (36% clay, 6% organic carbon) and could only be leached with difficulty from sandy and silty loams. Even from the silty loam, less than 0.05% of the total quantity of Aroclor 1016 added

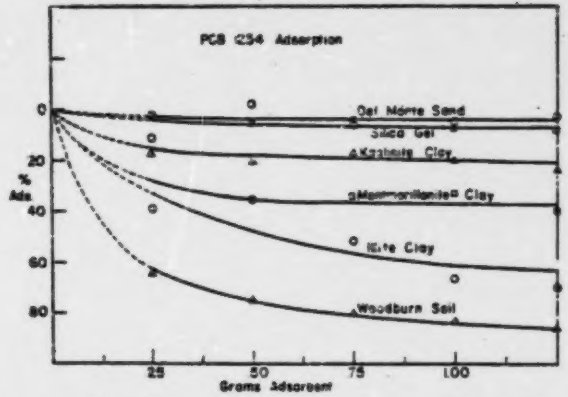


Plot of log E.M. (ecological magnification) for fish *vs.* log octanol/water partition coefficient.



Plot of log E.M. (ecological magnification) for fish *vs.* log water solubility (ppb).

Figure II.6.6 (from ref. 45)



Percent decrease in the concentration of Aroclor 1254 by the addition of increasing amounts of adsorbent. Original concentration of Aroclor 1254, 56 ppb

Figure II.6.7 (from ref. 37)

to the soil was leached in a four-month period. It was also observed in this experiment that the material leached from the soil consisted mainly of mono- and dichlorobiphenyls: the tetrachlorobiphenyls were apparently retained completely by the soil particles (Figure II.6.8).

In aquatic environments, PCBs are strongly associated with sediments and are usually found at much higher concentrations in sediments than in water in contact with them (12, 48, 49). Munson _et_ _al_. (50) have found that PCBs (and other chlorinated hydrocarbons) in Chesapeake Bay are strongly associated with suspended sediments (Figure II.6.9): the PCBs are believed to be transported into the Bay from rivers and harbors and to move around the Bay in association with the suspended sediments (50). As with other chlorinated hydrocarbons (51), PCBs are probably associated particularly strongly with micro-particulates (down to 0.15 μm in diameter or less).*

There has been some debate whether PCBs carried in the air are in the vapor phase or are attached to airborne particulates. Recent measurements of PCBs in air over the North Atlantic Ocean (52, 53) have suggested that most of the PCBs there are in the vapor phase. However, PCBs have been detected on airborne particulates in urban areas (2) and on particulate matter in dry fallout (12, 54). It seems likely that there is some differentiation of the chlorobiphenyls, because the PCBs detected as vapors over the North Atlantic were reported as matching Aroclors 1242 and 1248 (tri- and tetrachlorobiphenyls) while those on dry fallout were reported as matching Aroclor 1254 and Clophen A50 (penta- and higher chlorobiphenyls) (12, 53, 54). However, the measurements are not definitive, because some of the differen-

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* In the study reported in ref. 51, the chlorinated hydrocarbons extracted from the micro-particulate fraction of suspended sediments in water from Lake Erie were identified as various pesticides, including aldrin and endrin. However, this study took place before the recognition of PCBs as widespread contaminants in the aquatic environment, and it is likely that at least some of the residues in fact consisted of PCBs.

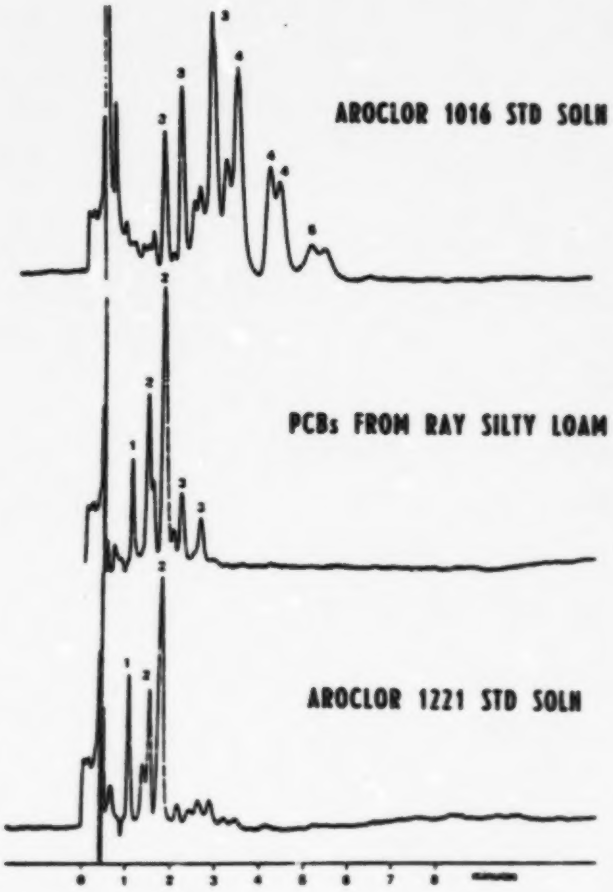
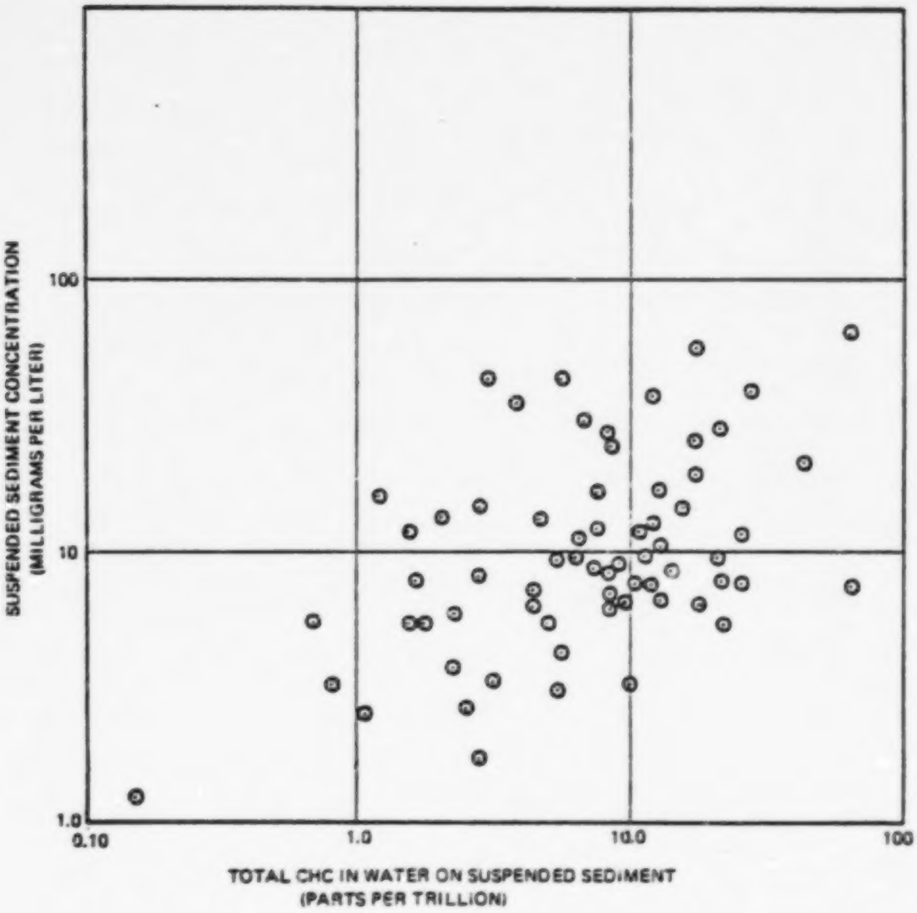


Figure II.6.8 (from ref. 47). Gas chromatograms illustrating differential leaching of components of Aroclor 1016 from soil.



A plot of the log of the suspended sediment concentration in the water (milligrams per liter) versus the log of the total CHC concentration in the water associated with the suspended sediment (parts per trillion).

Figure II.6.9 (from ref. 50)

tiation may take place during the process of collection -- i.e., the more volatile lower chlorobiphenyls may evaporate from the particulates as they are trapped on the collectors.*

II.6.6 Concentration at the air-water interface

Two recent studies have indicated that PCBs, like other chlorinated hydrocarbons, are strongly concentrated into the surface microlayers of ocean and estuarine waters, especially in surface slicks of natural lipids or mineral oils. Table II.6.7 summarizes two sets of measurements made in Narragansett Bay, Rhode Island (55). Sample 1 was from a heavy slick and Sample 2 from a less visible slick. Table II.6.8 summarizes a series of measurements in the Sargasso Sea (52). In each case the surface waters that were sampled contained concentrations of PCBs 3-50 times higher than the sub-surface waters. Since, for practical reasons, the surface microlayer itself could not be sampled, it is likely that the enrichment factor in the microlayer is often very much higher than this.

There appears to be some differentiation of chlorobiphenyls at the air-water interface, since Bidleman and Olney (52) reported that airborne PCBs resembled Aroclors 1242 or 1248 while those in the surface waters resembled Aroclors 1254 or 1260. However, it is not clear whether this differentiation results from differential volatility, differential fallout, or some other mechanism. Indeed, there are several mechanisms by which PCBs can be transferred across the air-water interface, and it is not yet clear for either inland waters or the oceans whether the net transfer is upwards or downwards (56).

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* Thomas has recently detected PCBs in both gaseous and particulate fractions of ambient air, in a ratio of about 5:1 (396). Similar results have been reported by Tzou (402).

Table II.6.7 (from ref. 55)

CONCENTRATION AND ENRICHMENT FACTORS OF POLYCHLORINATED BIPHENYLS (AS AROCLOR 1254) IN SURFACE MICROLAYER SAMPLES FROM NARRAGANSETT BAY, RHODE ISLAND

Sample 1			Sample 2		
Concentration (µg/liter)		Enrichment factor	Concentration (µg/liter)		Enrichment factor
Surface	Subsurface		Surface	Subsurface	
4.2 1	0.15 ± 0.04	28±10	0.45 ± 0.11	≤0.05	≥ 9

Table II.6.8 (from ref. 52)

POLYCHLORINATED BIPHENYLS (AS AROCLOR 1260) IN SARGASSO SURFACE MICROLAYER (SM) AND SUBSURFACE WATER (SS)

Collection date (1973)	Location	Sample	PCB* (ng/liter)	Enrichment factor
4/9	29°56'N,64°40'W	SM	11.2	3.1
		SS	3.6	
4/10	30°45'N,66°50'W	SM	4.9	5.4
		SS	<0.9	
4/10	30°34'N,66°59'W	SM	8.3	8.3
		SS	1.0	
4/11	28°53'N,65°07'W	SM	42.6**, 19.3	47.3
		SS	<0.9**, <0.9	21.4
4/12	29°56'N,63°00'W	SM	3.8	4.2
		SS	<0.9	
4/13	30°00'N,64°30'W	SM	5.6	3.5
		SS	1.6	
4/16	31°34'N,63°49'W	SM	5.0	2.8
		SS	1.8	
4/17	31°38'N,63°57'W	SM	8.4	9.3
		SS	<0.9	

*Blank value was 0.9 and was subtracted in calculating the above results.

**Sample taken in a Sargassum windrow.

II.7 Physical properties of chlorinated dibenzofurans

No measurements appear to have been published of the physical properties of CDFs that would be required to predict their environmental behavior. Tetra- and pentachlorodibenzofurans are more polar than the corresponding chlorobiphenyls (31), but their retention times in gas-liquid chromatography are similar (36).

II.8 Chemical properties of chlorobiphenyls and commercial mixtures

II.8.1 General chemical stability

As a general rule, PCBs are considered to be inert to almost all of the typical chemical reactions. PCBs do not undergo oxidation, reduction, addition, elimination, or electrophilic substitution reactions except under extreme conditions. For example, whereas many compounds undergo oxidation with oxidizing reagents as mild as iodine and household hydrogen peroxide, mono-, di-, and trichlorobiphenyls require boiling nitric acid (4). Chlorines can be replaced by reductive dechlorination with any metal hydride such as lithium aluminum hydride while high temperature (245°) treatment with alkali in an autoclave can result in chlorine displacement by hydroxide.

II.8.2 Photochemical reactions

The reactions of paramount environmental importance are the few that PCBs appear to undergo readily. These are alkali- and photochemically-catalized nucleophilic substitutions and photochemical free radical substitutions, all of which occur with alkali and water.

Photolysis generally has been found to give three types of products depending on conditions (4, 57-61). Chlorine is replaced by hydrogen (reductive dechlorination) in non-aqueous, non-polar solvents and by alkoxy groups in non-aqueous, polar solvents. Chlorine is replaced by hydroxy groups in aqueous systems and in thin films on glass interfacing with aqueous systems. Studies in non-aqueous solvents do not appear to be environmentally significant in spite of the presence of analogous hydrogen donors in nature unless the PCB product with one less chlorine is found to be more toxic than the PCB reactant. Nevertheless, some useful information can be obtained on approximate rate of decomposition and site of chlorine loss from non-aqueous studies. In hexane, the most prominent reactions of 2,5,2',5'-tetrachlorobiphenyl were the progressive reductive dechlorination and formation of polymeric materials. Photolysis in hexane also showed 33 percent decomposition of the PCB in 24 hours. Ruzo et al. (57-59) have performed the most extensive and thorough studies of the photochemical decomposition of PCBs. They observed the reactions of the tetrachlorobiphenyls shown in Table II.8.1. It was found that 90 to 95 percent of the starting materials reacted in methanol solution. Products consisted predominantly of dechlorinated PCB with minor but detectable amounts of methanol substitution (methoxylated) products. In all cases, the amount of methoxylated products did not exceed five percent of the total amount of dechlorination products formed, with trichloromethoxybiphenyls being the main components. Methanol substitution occurred at the same site from which a chlorine was lost. The methoxylated product of 2,4,2',4'-tetrachlorobiphenyl was identified as 2,4,4'-trichloro-2' methoxybiphenyl by comparison of GC retention time and mass spectra with those of authentic samples prepared in the laboratory. Each of the tetrachlorobiphenyls which contained chlorines in the ortho position lost them upon irradiation. Similarly, those tetrachlorobiphenyls containing only meta- and para-chlorines upon irradiation lost the meta-chlorines preferentially. This behavior was also observed in the formation of the secondary products, dichlorobiphenyls, resulting in loss of ortho- or meta- but not

Table II.8.1 (from ref. 58)

PHOTOPRODUCTS IN HEXANE AND IN METHANOL

Tetrachlorobiphenyls	Dechlorinated products	Time, sec	Methoxylated products
I: 2,5,2',5'-	2,3',5-Trichlorobiphenyl 3,3'-Dichlorobiphenyl 3-Chlorobiphenyl*	167 128	Trichloromethoxybiphenyl Dichlorodimethoxybiphenyl*
II: 2,4,2',4'-	2,4,4'-Trichlorobiphenyl 4,4'-Dichlorobiphenyl 4-Chlorobiphenyl*	190 148	Trichloromethoxybiphenyl Dichlorodimethoxybiphenyl*
III: 2,3,2',3'-	2,3,3'-Trichlorobiphenyl 3,3'-Dichlorobiphenyl	182 129	Trichloromethoxybiphenyl Dichlorodimethoxybiphenyl*
IV: 3,4,3',4'-**	3,4,4'-Trichlorobiphenyl 4,4'-Dichlorobiphenyl	192 147	Trichloromethoxybiphenyl
V: 3,5,3',5'-	3,3',5-Trichlorobiphenyl*	181	
VI: 2,6,2',6'-	2,2',6-Trichlorobiphenyl* 2,2'-Dichlorobiphenyl*	158 69	Trichloromethoxybiphenyl*

*Represented less than 1% of total product formation.
**3,3',4-Tetrachlorobiphenyl was observed in <2%.

para-chlorines. The tertiary products, monochlorobiphenyls, were only formed in one percent yield or not at all. This occurred either because of insufficient energy absorption by dichlorobiphenyls at the wavelengths employed or by decreased reactivity of meta- and para-chlorines relative to those on the ortho position.

The rate of photolysis reaction for all PCBs studied was found to be zero order (independent of the amount of each PCB) in both hexane and methanol (Table II.8.2 -- ref. 58). A marked increase in rate was observed, however, when solutions were degassed prior to irradiation. Oxygen is known to act as a triplet (free radical) quencher by accepting or sponging up excess electronic energy from free radicals before any chemical change occurs. Thus, an increase in rate upon removal of oxygen implies that a free radical process is occurring. In the environment, this implies that photochemical transformation will be enhanced under anaerobic conditions.

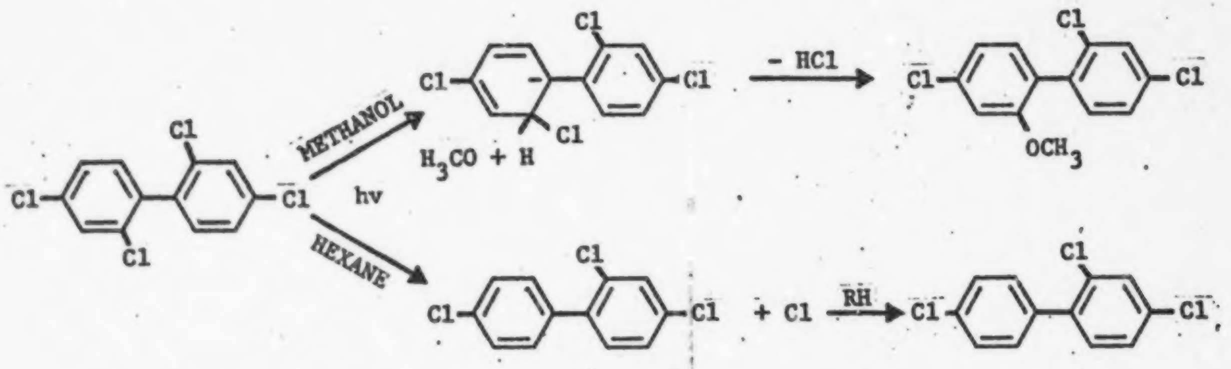
The two pathways operating to give replacement of chlorine by hydrogen and methoxyl group and the probable intermediates, inferring the corresponding mechanism, are shown in Figure II.8.1.

The photochemical behavior of higher chlorobiphenyls appears similar to that of the tetrachlorobiphenyls (60-61). 2,4,5,2',4',5'-hexachlorobiphenyl undergoes reductive dechlorination when irradiated in degassed methanol, losing one or both _ortho_-chlorines (61). Irradiation of Aroclor 1254 in aqueous solution gave rise to dechlorinated and hydroxylated products (60). Hexa- and octachlorobiphenyls are more photochemically reactive than tetrachlorobiphenyls (60), so that under irradiation the higher components of Aroclor 1254 are selectively degraded (60, 61 -- Table II.8.3).

Table II.8.2 Photolysis rates of tetrachlorobiphenyls (from ref. 58)

Tetrachlorobiphenyl	$k_h \times 10^8$ M sec^{-1} (degassed)*	$k_h \times 10^8$ M sec^{-1} (O_2)	$k_m \times 10^8$ M sec^{-1} (degassed)	$k_m \times 10^8$ M sec^{-1} (O_2)
2,4,2',4'-	15.0	2.8	18.0	4.2
2,3,2',3'-	2.4	1.0	4.0	1.5
2,5,2',5'-	2.2	0.5	4.8	0.7
3,4,3',4'-	1.4	0.8	3.5	2.2
2,6,2',6'-	0.4	0.2	0.5	0.2
3,5,3',5'-	<0.1		<0.1	

* Rate of reaction in hexane solution.



UV-irradiation of 2,2',4,4'-tetrachlorobiphenyl in methanol and hexane, products formed and probable mechanism.

Figure II.8.1 (from data in ref. 58)

TABLE II.8.3 (from ref. 61)

Sunlight Irradiation of Aroclor 1254.

Initial Concentration of Aroclor 1254 in Hexane

	1 ppm			10 ppm			100 ppm		
	% remaining after			% remaining after			% remaining after		
Peak No.	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks
1	97	109	143	65	104	104	85	131	139
2	111	137	161	111	140	180	114	164	184
3	74	76	79	113	94	88	110	110	101
4	83	104	109	127	108	143	92	99	120
5	103	89	87	98	96	94	118	70	63
6	181	211	196	210	206	241	157	205	218
7	58	51	36	68	58	40	68	58	42
8	84	89	75	111	98	90	105	96	80
9	66	47	30	53	44	44	80	56	47
10	62	47	41	49	50	59	80	64	54

II.8.3 Photochemical production and degradation of chlorinated dibenzofurans

The creation of free radicals by sunlight allows the environmental replacement of chlorines by hydroxy groups from water without the intervention of alkali. When this occurs at the ortho position (found to be the most preferred for chlorine loss) the resulting 2-hydroxychlorobiphenyl is perfectly positioned to allow oxygen to bond to an ortho position of the other ring. This results in the creation of potentially the most important class of contaminant in PCBs or commercial mixtures, the chlorodibenzofurans (CDFs).

Studies in aqueous solvents have continuously established the hydroxylation of mixtures of PCBs, although at times the location has not been established. Thus, irradiation of Aroclor 1254 in hydroxylic solvents at pH 9 yields compounds corresponding to the addition of water to the PCB molecules and a more polar carboxylic acid fraction. The same sample irradiated as a thin film does not yield the water-addition products, but mass spectrometry of 2,5,2',5'-tetrachlorobiphenyl reveals the presence of new hydroxylated species and a polar "carboxylic" fraction. No attempt was made to determine whether the polar carboxylic acid fractions in these studies were the same but it was generally concluded from a number of different irradiations of 2,5,2',5'-tetrachlorobiphenyl that dechlorination, formation of polymers, and carboxylic products, as well as hydroxylation, does occur (60).

Activating the ring will make it more capable of hydroxylation. Many means can be used to activate the ring, such as the intermediacy of a copper complex (the Ullmann reaction). In the presence of powdered copper or copper salts, a few hours at 160° converts normally unreactive o-chlorophenols to chlorinated dibenzo-p-dioxins, contaminants of 2,4-D and 2,4,5-T production with very similar chemical, physical and, probably, toxicological properties to CDFs (62, 63). Thus, in the environment either heat, light, or metals and metal salts in water could theoretically accelerate the transformation of PCBs into PCDFs.

The ultraviolet component of sunlight is sufficiently energetic to generate free radicals from both phenols and PCBs. The energies required to break the Ar-Cl bond to form hydroxy-PCBs and the ArO-H bond to form CDFs correspond to wavelengths near 360 and 320 nm, respectively -- clearly within the sunlight region. Analogous free-radical mechanisms have been proposed to explain reactions of polychlorophenol (PCP) and other chlorophenols in sunlight (62).

The alternate photonucleophilic formation of phenols from PCBs was demonstrated to afford hydroxy PCBs both with simple models and with highly complex Aroclors. Contrary to previous results (57), irradiation of an aqueous suspension of 4,4'-dichlorobiphenyl provided 4-chloro-4'-hydroxybiphenyl and 4-chlorobiphenyl (62, 63). Irradiation experiments with five pure 2-chlorinated biphenyls as 5 mg/l aqueous suspensions, showed that traces of 2-chlorodibenzofuran were detectable although only the 2,5-dichloro- and 2,5,2',5'-tetrachlorobiphenyls provided identifiable amounts (a roughly steady 0.2 percent yield during a seven-day irradiation) (62, 63). The environmental significance of this is threefold: (1) ortho-chlorobiphenyls can be hydroxylated by radiation similar to sunlight when they are suspended in aqueous media; (2) the product(s) are converted to CDFs; (3) rates of CDF formation by this process are approximately the same as their rates of degradation, leading to an approximately steady concentration. Hutzinger also has speculated that chlorodibenzofurans may be formed from chlorobiphenyls under photochemical conditions which lead to oxygenated products (60).

Although no chlorodibenzofurans were detected in a number of chlorobiphenyl samples which had been exposed to sunlight for over two months, preliminary results indicated the formation of chlorodibenzofurans from 2,4,6,2',5',6'-hexachlorobiphenyl in model experiments (irradiation in methanol) (Andersson et al., cited in ref. 64).

Before any knowledge on accumulation of CDFs can be obtained, relative rates of their formation and decomposition must be determined. Unfortunately, only a few rates of decomposition have been determined, such as those of 2,8-di- and octachlorodibenzofuran, as shown in Figure II.8.2 (64). Decomposition is faster in methanol than in hexane. In contrast to the chlorinated dibenzo-p-dioxins, where the degradation of the octachloro derivative on irradiation in methanol solution is much slower than that of the 2,7-dibenzo-p-dioxin, the di- and octachlorodibenzofurans show similar rates of decomposition.

Hutzinger et al. (64) observed that photolysis of 2,8-di- and octachlorodibenzofuran in methanol and hexane solutions results in rapid dechlorination and accumulation of unidentified resinous polymeric products. Dechlorination is also observed to a certain extent when thin films of these compounds were exposed to sunlight. This is similar to the non-aqueous behavior of PCBs from which these CDFs arose. This suggests that CDFs in the environment, like PCBs, will only reductively dechlorinate when in organic media or thin films. Crosby and Moilanen (63) found that irradiation of 2,8-dichlorodibenzofuran resulted in a slow dehalogenation in hexane, explaining the previous appearance of 2-chloro-dibenzofuran. Irradiation in methanol substantiated the rapid disappearance of the dichlorodibenzofuran (64). Decomposition in aqueous suspension was found to be slow (63).

Unfortunately, these preliminary data do not allow direct comparison of environmental dibenzofuran degradation rates with rates of their photo-

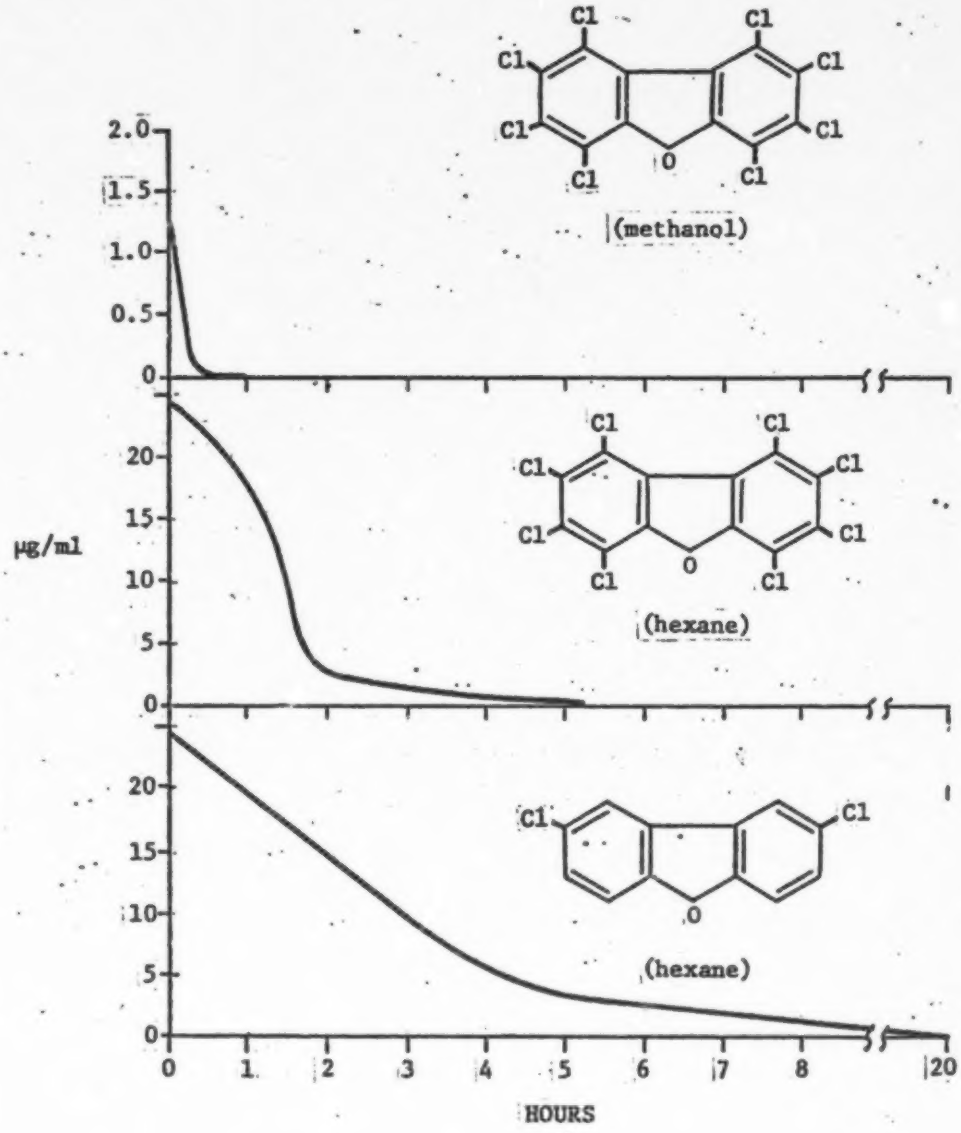


Figure II.8.2 (from ref. 64)

Photochemical degradation of chlorodibenzofurans in solution. Irradiation wavelength 310 nm.

chemical formation from corresponding chlorobiphenyls. In view of the photochemical lability observed for some chlorodibenzofurans, however, it seems reasonable that most isomers will not accumulate excessively in the environment with the possible exception of 2-CDF. Since accumulation is relative when a wide range of toxicity is considered, the steady state conversion of two-tenths percent of some PCBs to PCDFs is very important and potentially very significant. A further environmental complication is that other investigators have been unsuccessful in detecting the conversion of PCBs to PCDFs in light, perhaps due to the aforementioned rapid decomposition for most isomers under energetic (254 nm) irradiation conditions or the presence of sensitizers. The addition of the photosensitizer 4,4'-dichlorobenzophenone to a methanol solution of 2,8-dichlorodibenzofuran during irradiation resulted in a sharp increase in the decomposition rate of that CDF (63). Both natural and synthetic photosensitizers have been shown to affect other xenobiotics, and the environmental sensitization of degradative photolysis of CDFs seems quite plausible.

II.8.4 Probable formation of PCDFs from PCBs in service.

In the "Yusho" incident in Japan in 1968, large numbers of persons were poisoned by consuming rice-oil contaminated with PCBs (Kanechlor KC-400) from a leaking heat-exchanger (35; see Section III.15 below). Recent studies have shown that although the original Kanechlor KC-400 contained only about 20 ppm of PCDFs, the toxic rice-oil contained relatively much larger quantities of PCDFs (PCB/PCDF ratio 84-200 in the rice-oil, versus 50,000 in KC-400) (34; 35, Table 5). Further, the PCDFs in the Yusho oil contained substantial quantities of hexachlorodibenzofurans, which have not been detected in KC-400 (33-35). This suggests that most of the PCDFs in the Yusho oil had been formed in service, probably in the heat-exchanger. According to Kuratsune, elevated levels of PCDFs have been discovered in PCBs in other heat-exchangers in Japan.*

In a parallel incident in the United States in 1971, fish meal being processed for poultry food was contaminated with Aroclor 1242 and proved highly toxic to broiler chickens (65). Subsequent tests with the contaminated feed, which contained 148 ppm of PCBs, showed it to be much more toxic to chicks than feed containing 200 or even 400 ppm of Aroclor 1242, and the symptoms of poisoning were different (2, 65: see Section III.6.2 below). This again suggests that a more toxic agent had been formed in service. This situation is complicated by the report that the contaminated feed also included 8.8 ppm of "BHC" (presumably an isomer of HCH, hexachlorocyclohexane) (65). However, the identification of this compound is questionable, because HCH is rarely found in marine fish or in other components of poultry feeds at more than trace levels.

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* This information was presented by Kuratsune at the PCB conference in November 1975 (9) but documentation is not yet available.

Hence the component reported as BHC in the contaminated fish meal may in fact have been a breakdown product of PCBs.

According to Broadhurst (66), a general disadvantage of PCBs in many of their applications (including capacitor and transformer uses as well as heat transfer uses) is their tendency to decompose under the action of heat or electrical arcing to form corrosive HCl. Hence the evidence summarized above, that PCDFs or other toxic breakdown products may be formed in service in heat-exchangers, needs to be followed up by investigation of the composition and toxicity of PCBs after service in electrical equipment also (2).

III. TOXIC EFFECTS

III. 1. Introduction

Toxicological aspects of PCBs have been reviewed in refs. 1, 2 and 5, and in a number of papers presented at the National Conference on Polychlorinated Biphenyls in November 1975 (9, 16, 29, 35, 67-75). The following summary is concerned especially with three aspects of the toxicology of PCBs:

1. Definition of the toxic effects which have been observed reliably at low levels of exposure to PCBs; these are of greatest importance in establishing criteria.

2. Review of the differences in toxicity between the various commercial mixtures, which reflect differences in toxicity among the individual chlorobiphenyls.

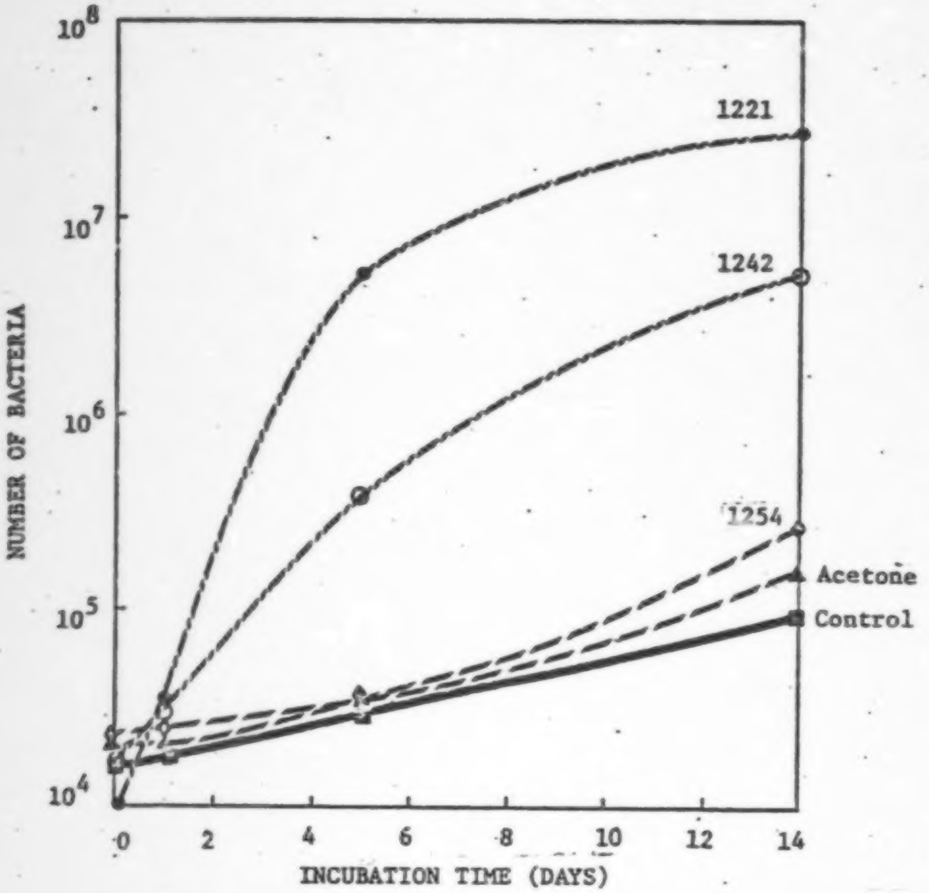
3. Elucidation of the role of PCDFs in the observed toxicity of commercial mixtures or environmental residues.

III. 2. Effects on microbial systems.

The effects of PCBs on micro-organisms range from stimulus to inhibition of growth, depending upon the degree of chlorination, the type of organism, and other environmental factors. When Aroclors 1221, 1242, and 1254 were added to bacteria from lake water (including Achromobacter sp. and Pseudomonas sp.) in 1 percent glucose medium, no inhibition of growth occurred at concentrations up to 0.1 percent Aroclor. In fact, there was a slight stimulation by Aroclors 1221 and 1242 (76). Lake water samples incubated with Aroclors as the carbon and energy sources illustrated that bacteria could use 0.05 percent Aroclors 1221 and 1242 for growth support (see Figure III.2.1) but not Aroclor 1254 (76). The ability of the bacteria to utilize PCBs decreased with an increase in percent of chlorination.

When natural waters were enriched by the addition of 0.1 ppm Aroclor 1260, bacterial counts yielded increasing numbers of viable bacteria (41). No adverse effects were noted, with growth of the treated cells exceeding that of the control (see Table III.2.1). In spite of the slight growth enhancement, no metabolites were detected and unaltered parent compounds were recovered.

Isolates of estuarine bacteria (including _Flavobacterium_, _Bacillus_, _Corynebacterium_, _Pseudomonas_, _Achromobacter_, _Micrococcus_, and _Serratia_) were grown on agar with Aroclor 1016 and 1242 saturated paper discs (77). Of 85 tested cultures, 26 were inhibited to varying extents by 0.5 mg (5 mg in 0.1 ml acetone) of either Aroclor. Cultures sensitive to Aroclor 1242 were also sensitive to Aroclor 1016, and 65 percent of the cultures were still sensitive at 0.1 mg. Concentrations of 0.1 mg Aroclor 1016 inhibited 58 percent of the cultures sensitive at 0.5 mg. Four representative cultures of the sensitive bacteria (two gram-positive and two gram-negative) were tested for growth inhibition in liquid culture with 10 μg/ml (10 ppm) of Aroclor 1242. The extended lag times suggest an adaptation mechanism (see Figure III.2.2) and possibly extended time (greater than the 24 hours used) on agar would produce similar results. Previous reports indicate that gram-positive bacterial growth was inhibited by organochlorine insecticides (chlordane) and gram-negative bacteria were unaffected (77). The bacteria sensitive to Aroclor 1254 were both



The time/growth curve of bacteria in 0.05% Aroclor 1221, Aroclor 1242, and Aroclor 1254 as carbon and energy sources.

Figure III.2.1 (from ref. 76)

Table III.2.1 (from f. 41)

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from the Fraser River, held in the laboratory for 12 weeks at 7°C

Time, weeks	Compound	Bacteria/ml	Recovery, %	
			Water	Glass wool
0	α-Chlordane	1.8×10^{2}	98.7	0[a]
6	α-Chlordane	1.3×10^{4}	59.8	9.5
6	α-Chlordane	2.3×10^{4}	59.7	17.7
12	α-Chlordane	4.2×10^{5}	41.7	7.6
12	α-Chlordane	8.8×10^{4}	44.1	6.3
0	γ-Chlordane	1.8×10^{2}	90.6	0
6	γ-Chlordane	2.2×10^{4}	53.0	18.6
6	γ-Chlordane	2.5×10^{4}	55.6	6.4
12	γ-Chlordane	2.6×10^{5}	37.1	9.4
12	γ-Chlordane	7.1×10^{4}	38.1	3.4
0	Lindane	1.8×10^{2}	101.0	0
6	Lindane	1.2×10^{4}	99.7	0
6	Lindane	1.4×10^{4}	101.5	0
12	Lindane	5.3×10^{5}	96.7	0
12	Lindane	5.3×10^{4}	97.2	0
0	DDT	2.0×10^{2}	95.6	0
6	DDT	1.8×10^{4}	67.4	5.6
6	DDT	2.2×10^{4}	71.3	3.2
12	DDT	4.8×10^{4}	69.7	7.2
12	DDT	3.4×10^{4}	68.7	3.9
0	PCB[b]	1.9×10^{2}	95.6	0
6	PCB[b]	5.2×10^{4}	60.1	1.8
6	PCB[b]	2.7×10^{4}	60.9	4.5
12	PCB[b]	1.1×10^{5}	64.1	1.2
12	PCB[b]	—	74.7	5.5
0	Acetone only	2.2×10^{2}	0	0
6	Acetone only	9.3×10^{3}	0	0
12	Acetone only	4.6×10^{4}	0	0
0	Control	2.3×10^{2}	0	0
6	Control	7.1×10^{3}	0	0
12	Control	4.5×10^{3}	0	0

[a] No residue detected.
[b] 0.1 ppm Aroclor 1260.

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from the Nicomekl River, held in the laboratory for 12 weeks at 16°C

Time, weeks	Compound	Bacteria/ml	Recovery, %	
			Water	Glass wool
0	α-Chlordane	1.5×10^{4}	92.0	0[a]
6	α-Chlordane	—	50.2	0.8
6	α-Chlordane	—	51.1	1.5
12	α-Chlordane	1.1×10^{5}	20.5	1.2
12	α-Chlordane	5.7×10^{5}	16.6	0.8
0	γ-Chlordane	6.4×10^{3}	100.8	0
6	γ-Chlordane	—	34.0	0.2
6	γ-Chlordane	—	24.7	1.3
12	γ-Chlordane	1.1×10^{5}	2.3	0.5
12	γ-Chlordane	5.4×10^{4}	13.9	1.5
0	Lindane	8.5×10^{3}	98.4	0
6	Lindane	—	90.5	0
6	Lindane	—	88.5	0
12	Lindane	1.2×10^{4}	79.0	0
12	Lindane	4.0×10^{3}	76.0	0
0	DDT	1.5×10^{4}	96.3	0
6	DDT	—	34.4	2.2
6	DDT	—	43.7	3.4
12	DDT	9.9×10^{4}	30.1	1.1
12	DDT	1.4×10^{3}	23.6	3.5
0	PCB[b]	2.4×10^{4}	96.2	0
6	PCB[b]	—	73.8	1.4
6	PCB[b]	—	74.5	1.6
12	PCB[b]	7.5×10^{3}	33.0	1.1
12	PCB[b]	4.4×10^{3}	56.7	1.1
0	Acetone only	1.8×10^{4}	0	0
6	Acetone only	—	0	0
12	Acetone only	2.6×10^{4}	0	0
0	Control	4.9×10^{4}	0	0
6	Control	—	0	0
12	Control	1.4×10^{4}	0	0

[a] No residue detected.
[b] 0.1 ppm Aroclor 1260.

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from Georgia Strait, held in the laboratory for 12 weeks at 9°C

Time, weeks	Compound	Bacteria/ml	Recovery, %	
			Water	Glass wool
0	α-Chlordane	1.0×10^{2}	91.3	0[a]
6	α-Chlordane	1.1×10^{5}	41.9	0.8
6	α-Chlordane	8.9×10^{4}	30.3	1.0
12	α-Chlordane	3.0×10^{3}	50.7	0.6
12	α-Chlordane	1.3×10^{5}	53.2	0.6
0	γ-Chlordane	1.1×10^{2}	94.4	0
6	γ-Chlordane	9.3×10^{4}	31.9	21.5
6	γ-Chlordane	—	24.9	9.5
12	γ-Chlordane	4.5×10^{4}	28.4	9.6
12	γ-Chlordane	1.3×10^{4}	35.6	4.3
0	Lindane	8.6×10^{2}	99.9	0
6	Lindane	1.4×10^{5}	91.5	0
6	Lindane	6.0×10^{4}	96.0	0
12	Lindane	1.3×10^{4}	99.2	0
12	Lindane	1.1×10^{4}	90.5	0
0	DDT	8.0×10^{2}	90.8	0
6	DDT	5.8×10^{4}	65.8	3.2
6	DDT	7.2×10^{4}	49.3	17.2
12	DDT	1.1×10^{4}	53.0	6.0
12	DDT	1.9×10^{3}	36.6	10.7
0	PCB[b]	7.8×10^{2}	93.5	0
6	PCB[b]	9.8×10^{4}	70.7	1.3
6	PCB[b]	6.5×10^{4}	40.7	6.2
12	PCB[b]	3.5×10^{3}	37.7	2.4
12	PCB[b]	1.1×10^{3}	38.7	0.8
0	Acetone only	1.1×10^{3}	0	0
6	Acetone only	3.4×10^{4}	0	0
12	Acetone only	4.5×10^{3}	0	0
0	Control	8.0×10^{2}	0	0
6	Control	2.4×10^{5}	0	0
12	Control	5.1×10^{3}	0	0

[a] No residue detected.
[b] 0.1 ppm Aroclor 1260.

gram-positive and negative, with slightly more (61 percent) of the sensitive bacteria gram-positive. When PCB-sensitive and nonsensitive strains were compared, the biochemical activities of test bacteria fell into two different groups. Amylase was produced by 76 percent and gelatinase by 86 percent of the sensitive strains, whereas the nonsensitive strains showed only 33 percent and 42 percent activities, respectively. No conclusions were drawn by Bourquin and Cassidy (77) as to the possible significance of the prevalence of PCB-sensitivity among amylase and gelatinase producers. However, they suggested further investigation to determine any relationships to total nutrient catabolism. In another recent paper, Bourquin _et al._ (78) again reported little difference in activity between Aroclors 1242 and 1016. Results for 25 of the 100 cultures tested are shown in Table III.2.2.

Using _Escherichia coli_, a common human intestinal aerobe, Keil _et al_. (79) found that Aroclor 1242 did not inhibit growth at concentrations up to 1,000 ppm. Aroclor 1242 at a concentration of 0.1 ug/ml^{-1} (0.1 ppm), and possibly at 0.01 ug/ml^{-1} (0.01 ppm), stimulated _E_. _coli_ growth (see Table III.2.3).

In a separate experiment using _E_. _coli_ and _Bacteriodes fragilis_ (an intestinal anaerobe), Greer _et al_. (80) found no detectable effect on growth using 0.01 ppm (0.01 mg/l) and 0.1 ppm (0.1 mg/l) Aroclor 1242. However, there was an apparently dose-related depression of lipid synthesis in _B_. _fragilis_ only (see Table III.2.4).

The effect of 2,2'-dichlorobiphenyl on microbial predators of _E_. _coli_ was found to decrease kill rates at lower concentrations (81).

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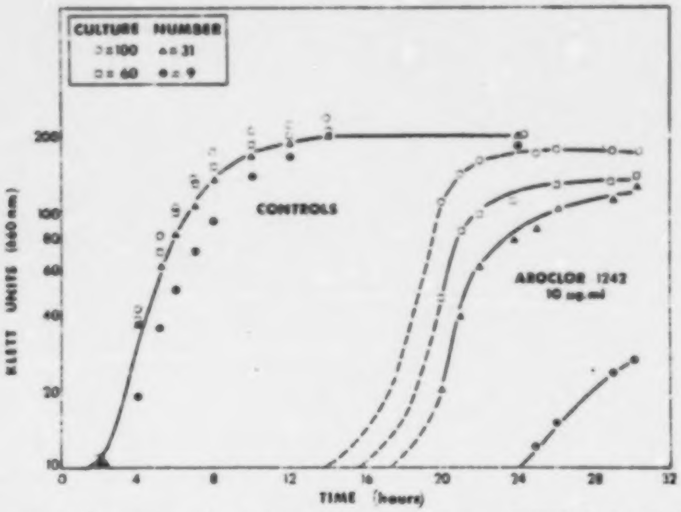


Four sensitive bacteria were tested for growth in one-half strength 2216 Marine Broth containing 10 µg of Aroclor 1242 per ml. Symbols: (○) Achromobacter sp., (●) Bacillus sp., (▲) Corynebacterium sp., and (□) Pseudomonas sp. Controls contain one-half strength 2216 Marine Broth only; all salinities were 20‰.

Figure III.2.2 (from ref. 77)

Table III.2.2 (from ref. 78)

Inhibition of growth of estuarine bacteria on nutrient seawater medium by PCB's

GBERL Culture No.	Gram Reaction & Morphology	Genus	Aroclor® 1242 (mg)			Aroclor® 1016 (mg)		
			0.1	0.25	0.5	0.1	0.25	0.5
3	+ ROD	Unknown	++	++	+++	++	++	+++
21	– ROD	Unknown	++	++	+++	++	++	+++
35	– ROD	*Flavobacterium* sp.	++	++	++	+	+++	+++
39	– COCCOID	Unknown	++	+++	+++	++	+++	+++
53	– ROD	Unknown	++	+++	+++	+++	+++	+++
54	+ ROD	*Bacillus* sp.	+++	+++	+++	+	+++	+++
7	+ ROD	*Bacillus* sp.	+	+	+	x	+	++
9	+ ROD	*Bacillus* sp.	+	++	+++	+	++	+++
31	– ROD	Unknown	+	+	+++	+	+	+++
60	+ ROD	*Bacillus* sp.	+	+	+	++	++	++
86	– ROD	*Flavobacterium* sp.	+	+	+	x	+	+
100	– ROD	*Pseudomonas* sp.	+	++	++	+	++	++
8	+ ROD	*Corynebacterium* sp.	x	+	++	x	+	++
11	– ROD	*Achromobacter* sp.	x	+	++	x	+	++
42	+ COCCUS	*Micrococcus* sp.	x	+	++	x	+	++
44	+ COCCUS	*Micrococcus* sp.	x	+	+	–	x	+
93	+ ROD	Unknown	x	+	+	–	x	+
43	+ COCCUS	*Micrococcus* sp.	–	+	++	–	+	++
5	– COCCOID	*Serratia* sp.	–	–	++	–	–	++
13	– ROD	*Achromobacter* sp.	–	–	++	–	–	++
28	– ROD	*Achromobacter* sp.	–	–	++	–	–	++
32	+ ROD	*Corynebacterium* sp.	–	+	+	–	x	+
41	– COCCOID	Unknown	–	–	++	–	–	++
67	– ROD	*Achromobacter* sp.	–	+	+	–	–	+
69	– ROD	Unknown	–	–	++	–	–	++

Degree of sensitivity: +++ (18-20 mm zone), ++ (16-18 mm), + (14-16 mm), x (slightly), – (not sensitive).

Table III.2.3 (from ref. 79)

LYOPHILIZED WEIGHTS AND TOXICANT UPTAKE BY ESCHERICHIA COLI CULTURES EXPOSED TO PCB

Treatment	Mean Harvest Weights (mg)	Mean Residue ($\mu g\ g^{-1}$)
Bacterial Series I		
PCB 0.1 $\mu g\ ml^{-1}$	86.0	13.6
PCB 0.01 $\mu g\ ml^{-1}$	90.4	4.4
Control	44.5	0
Acetone control	27.2	1.2
Uridine control	41.6	0
LSD_{05}*	13.6	10.8
Bacterial Series II		
PCB 0.1 $\mu g\ ml^{-1}$	186.4	
PCB 0.01 $\mu g\ ml^{-1}$	153.2	
Control	160.8	
Acetone control	133.6	
LSD_{05}*	23.6	

* LSD_{05} = Least significant at 95 percent probability level calculated from one way analysis of variance.

Table III.2.4 (from ref. 80)

HARVEST WEIGHTS, TOXICANT RESIDUE, AND LIPID YIELDS OF *BACTERIODES FRAGILIS* CULTURES TREATED WITH PCB

Treatment	No. Replicates	Mean Harvest Weight (mg)	Mean Toxicant Residues (µg)	Mean Lipid Yields (µg/mg dry wt)
.01 ppm PCB	4	137.4	N.D.[*]	23.2
.1 ppm PCB	2	210.4	N.D.[*]	16.2
Control	4	119.7	0	34.9
Acetone Control	4	109.9	0	35.7
LSD$_{05}$[**]	4/4	26.0		7.8
	2/4	31.8		9.5

---

[*] N.D. = Not Determined

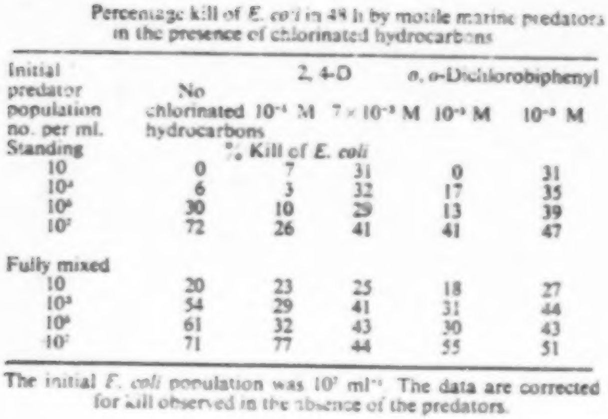
[**] LSD = Least significant difference at 95 percent probability level calculated from one way analysis of variance.

The predators (the dominant Spirillum spp. and a pseudomonad), were isolated from sea water using E. coli as the sole carbon source. Neither the predator nor E. coli viability and activity were affected by 10^{-3}M (200 ppm) 2,2'-dichlorobiphenyl. Table III.2.5 shows percentage kill of E. coli in the presence of varying predator populations and dichlorobiphenyl concentrations.

Walsh and Mitchell (81) noted that the lower dichlorobiphenyl concentrations (10^{-5}M) which generally decreased E. coli kill are similar to those used previously to inhibit chemotaxis in motile marine bacteria. The higher concentrations (10^{-3}M) which increased kill rates in the standing flasks for lower predator populations were similar to those Walsh and Mitchell observed to increase chemotaxis. The standing flasks probably contained a higher concentration of both microbes and chemicals at the surface (41). Thus, the changes in E. coli kill by microbial predators may be due to inhibition or excitation of the chemotactic response by dichlorobiphenyls.

PCBs may cause changes in the ecological role of microorganisms. The differing sensitivities of the bacteria to the PCBs present may cause changes in composition of community populations. For example, Escherichia coli appears relatively resistant to PCBs at the concentrations tested (79-81). Stimulation of E. coli growth occurred at lower concentrations (0.1 and 0.01 ppm) of Aroclor 1242 (79). Achromobacter sp. and Pseudomonas sp. also had growth slightly stimulated with 500 ppm Aroclors 1242 and 1221. Oloffs et al. (41) increased bacterial numbers in natural waters by the addition of 0.1 ppm Aroclor 1260. If, as Bourquin and Cassidy (77) reported, one-third of a natural

Table III.2.5 (from ref. 81)



Percentage kill of *E. coli* in 48 h by motile marine predators in the presence of chlorinated hydrocarbons

Initial predator population no. per ml.	No chlorinated hydrocarbons	2, 4-D 10^{-4} M	2, 4-D 7×10^{-3} M	*o*, *o*-Dichlorobiphenyl 10^{-6} M	*o*, *o*-Dichlorobiphenyl 10^{-3} M
	% Kill of *E. coli*				
Standing					
10	0	7	31	0	31
10^4	6	3	32	17	35
10^6	30	10	29	13	39
10^7	72	26	41	41	47
Fully mixed					
10	20	23	25	18	27
10^3	54	29	41	31	44
10^6	61	32	43	30	43
10^7	71	77	44	55	51

The initial *E. coli* population was 10^7 ml^{-1}. The data are corrected for kill observed in the absence of the predators.

estuarine bacterial population is inhibited by chlorinated biphenyls and stimulation of some of the remaining two-thirds is possible (41,79), with no response by other members, a totally different bacterial community may be created. Although the concentrations of PCBs required to show effects on single species cultures are much higher than those occurring in natural waters, they are comparable with those reported from sediments and sewage sludge (2,10-12, 48-50). Moreover, as in the case of phytoplankton (Section III.3), effects on mixed-species cultures may take place at lower concentrations of PCBs.

Changes in community composition, coupled with possible increased (or decreased) chemotactic responses of predatory micro-organisms (81) may cause further disruption of normal carbon cycling and pollution control associated with microbes. It may be necessary, in light of the effects of PCBs, to change some of the criteria presently used to determine water pollution. For example, low counts of *E. coli* may be indicative of high PCB levels stimulating predators, not clean water.

Natural adaptive mechanisms of micro-organisms may compound or counteract the effects of PCBs. Baxter *et al.* (82) stressed the adaptation of *Nocardia* spp. grown on biphenyl. The ability to degrade PCBs and the rate of degradation appear dependent upon degree of chlorination as well as available precursors. Thus, microbial communities may undergo continual changes or cycles as bacteria, chemical components and the associated processes and dependents fluctuate.

III.3 Effects on Phytoplankton

Phytoplankton are the photosynthetic organisms which form the basis for all marine and some fresh-water food-webs. A number of experiments have shown that PCBs interfere with photosynthetic mechanisms in certain species of phytoplankton at concentrations as low as 0.1 ppb (ug/l) or below (83-92). In most cases the effects measured were reductions in the uptake of radiolabelled carbon (supplied as bicarbonate), a measure of the rate of carbon fixation. In a recent assessment, Fisher (83) has suggested that the primary effects of PCBs are to reduce the rates of cell growth and cell division; the important consequences are likely to be changes in the species composition of marine phytoplankton communities, rather than a reduction in the total rate of photosynthesis by the community.

The radiocarbon uptake of two green algae, Scenedesmus quadricauda (fresh water) and Dunaliella tertiolecta (estuarine) was reduced by 60 percent and 35 percent, respectively, when they were exposed to Aroclor 1254 at 100 ppb (84). Effects on S. quadricauda were marked even at 0.1 ppb, the lowest concentration tested. (Table III.3.1). Inhibition was greater in Scenedesmus which possesses a cell wall, as opposed to the naked Dunaliella (see Table III.3.1). Since the cell wall appears not to prevent absorption, either the cell or chloroplast membrane would appear to be the selective barrier. There was no effect on radiocarbon uptake in either alga by DDT in concentrations up to 1 ppm. However, photoreduction of isolated chloroplasts was inhibited by both PCBs and DDT, suggesting that the cell membrane possesses an active transport site preferentially structured for PCB (84).

Table III.3.1 (from ref. 84)

INHIBITION OF CARBON-14 UPTAKE IN SCENEDESMUS AND DUNALIELLA BY CHLORINATED HYDROCARBONS

Concentration ppb	C^{14} Uptake, % Control DDT	 Aroclor 1242[a]	 Aroclor 1242[b]
0.1	90.66	79.81	113.2
1	111.44	70.45	94.1
10	98.85	74.02	103.6
10^2	109.12	40.39	65.7
10^3	101.74	34.53	56.9

[a] Scenedesmus

[b] Dunaliella

Source: Luard, E.J. 1973. Sensitivity of Dunaliella and Scenedesmus (Chlorophyceae) to chlorinated hydrocarbons. Phycologia 12(1/2): 29-33.

Exposure of the marine diatom Cylindrotheca closterium to 10 or 100 ppb Aroclor 1242 resulted in inhibition of growth and diminished levels of chlorophyll and RNA synthesis (Table III.3.2). Levels of DNA were not affected, implying selective action by the polychlorinated biphenyls on cell metabolism (85). The chlorophyll content per cell was similar in the treated and control cultures, suggesting that the primary effect of PCBs was to inhibit cell growth and division rather than to inhibit photosynthesis per se. A similar result was obtained by Fisher (83), using two other diatom species exposed to 10 ppb Aroclor 1254.

Sensitivity of two marine diatoms Thallassiosira pseudonana and Rhizosolenia setigera to Aroclor 1254 increased with decreasing temperature (Table III.3.3)(87). R. setigera had growth completely stopped for 48 hours at concentrations of 0.1 to 10 ppb Aroclor 1254, after which growth resumed with less inhibition at the higher temperature (87: Figure III.3.1). T. pseudonana was only sensitive at 10 ppb Aroclor 1254. The effects of temperature, Aroclor 1254, and the interaction between temperature and Aroclor 1254 were all significant ($P < 0.001$), indicating that sensitivity to PCBs was dependent on temperature. The increased inhibition at temperatures at which cell division rates were slowest illustrates the narrowing of a sensitive species' niche with environmental stress. Thus, species more vulnerable to stress would be less capable of successful competition for resources. If this were true, species adapted to stressful conditions, such as occur in estuarine environments with salinity changes, would be expected to withstand higher levels of PCBs. Supporting this hypothesis is the finding

Table III.3.2 (from ref. 85)

HARVEST WEIGHT, CELL COUNTS, NUCLEIC ACID LEVELS, AND CHLOROPHYLL INDEX OF CYLINDROTHECA CLOSTERIUM CULTURES EXPOSED TO AROCLOR 1242

Treatments, Aroclor 1242	Harvest Weight (mg)	Final Cell Count ($\times 10^4$)	Chlorophyll Index (OD @ 670)	RNA (mg/mg)	PCB Levels (ppm)
PCB .1 ppm	11.5**	16.5**	.27**	.015**	109.2**
PCB .01 ppm	38.1	113.7	1.26	.033*	4.7*
Control	30.9	116.8	1.52	.039	0
Acetone Control	33.2	98.5	1.37	.040	0

Significantly different from control at .05 level

**Significantly different from control at .01 level

Source: Keil, J.E., L.E. Priester and S.H. Sandifer. 1971. Polychlorinated biphenyl (Aroclor 1242): Effects of uptake on growth, nucleic acids, and chlorophyll of a marine diatom. Bulletin of Environmental Contamination and Toxicology 6(2):156-159.

Table III.3.3 (from ref. 86)

GROWTH OF *T. pseudonana* AND *D. tertiolecta* AT THREE TEMPERATURES AND THREE CONCENTRATIONS OF PCBS. GROWTH RATES (μ) ARE EXPRESSED AS DIVISIONS PER DAY AND WERE CALCULATED ACCORDING TO THE EXPRESSION $\mu = (35)(\ln n_t - \ln n_{t0})/(t - t_0)$ WHERE $t - t_0$ REPRESENTS ELAPSED TIME IN HOURS AND n_t AND n_{t0} ARE THE POPULATION DENSITIES AT TIMES t AND t_0, RESPECTIVELY (EPPLEY & STRICKLAND, 1968). THE VALUES SHOWN (MEANS OF THREE REPLICATES) ARE GROWTH RATES FOR THE FIRST 72 H AT 18°C AND 25°C AND FOR THE FIRST 120 H AT 12°C.

Temperature	*[PCB] in ptm*	*Growth rate of* T. pseudonana	*% control*	*Growth rate of* D. tertiolecta	*% control*
12°C	0·0	1·43	100	0·74	100
12°C	0·1	1·57	109	0·75	101
12°C	10·0	0·60	42	0·79	107
18°C	0·0	2·34	100	1·22	100
18°C	0·1	2·15	84	1·23	101
18°C	10·0	1·31	56	1·18	97
25°C	0·0	2·57	100	1·66	100
25°C	0·1	2·59	101	1·66	100
25°C	10·0	2·13	83	1·69	102

GROWTH OF *R. setigera* AT TWO TEMPERATURES AND FOUR CONCENTRATIONS OF PCBS. GROWTH RATES ARE EXPRESSED AS DIVISIONS PER DAY AND WERE CALCULATED AS SHOWN IN TABLE 1. THE VALUES SHOWN (MEANS OF THREE REPLICATES) ARE GROWTH RATES FOR THE FIRST 192 H AT 10°C AND FOR THE FIRST 144 H AT 15°C.

Temperature	*[PCB] in ptm*	*Growth rate of* R. setigera	*% control*
10°C	0·0	0·60	100
10°C	0·1	0·49	82
10°C	1·0	0·46	77
10°C	10·0	0·49	82
15°C	0·0	0·86	100
15°C	0·1	0·81	95
15°C	1·0	0·75	87
15°C	10·0	0·73	85

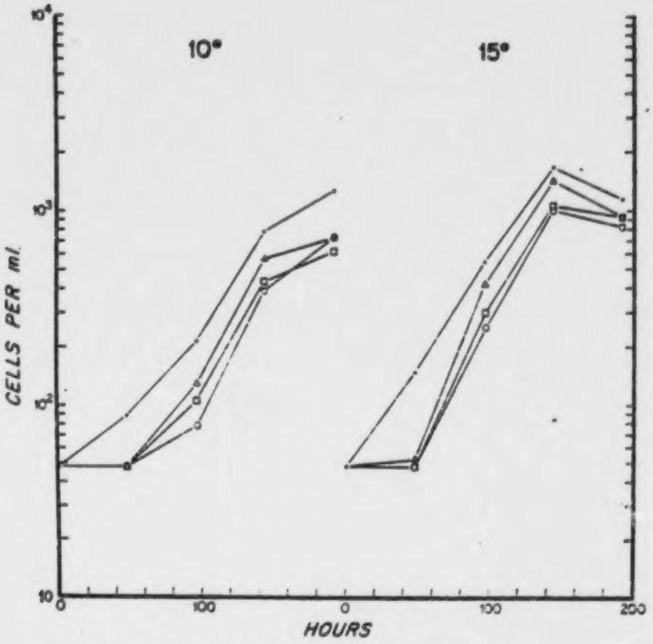


Fig. 2. Growth of *R. setigera* at two different temperatures and with four PCB concentrations. (●——●) = 0 ptm of PCBs; (Δ——Δ) = 0·1 ptm; (□——□) = 1·0 ptm; (○——○) = 10 ptm. Data points are the means of three replicates.

Fig. III.3.1 (from ref. 86)

that Dunaliella tertiolecta, an estuarine and marine green alga, was not affected at any of the temperatures or concentrations of Aroclor 1254 that inhibited the marine diatoms (86: Table III.3.3). Mosser et al. (87,88) also found differences in sensitivity to Aroclor 1254 between marine diatoms and the green alga D. tertiolecta. This alga was not affected by concentrations of Aroclor 1254 up to 1,000 ppb, while 10 ppb and 25 ppb affected the diatoms Skeletonema costatum and Thalassiosira pseudonana, respectively (see Figure III.3.2). The viability of T. pseudonana showed a decrease at 50 and 100 ppb Aroclor 1254. The sensitivity levels should be considered as maxima, since evaporation loss and adsorption of the PCBs to the walls of the flask have not been taken into account.*

Further investigations of geographic differences in sensitivity to PCBs compared estuarine and oceanic colonies of the same diatom species (89). Three species of diatoms were isolated from the relatively stable Sargasso Sea and from the more variable environments of continental shelf and estuarine areas. The diatoms tested were Thalassiosira pseudonana, Fragilaria pinnata and Bellerochia sp. All clones grew more slowly with 10 ppb Aroclor 1254 added, but clones from the Sargasso Sea were more sensitive than estuarine and continental shelf isolates (Table III.3.4). The clone from the continental shelf was, in turn, more sensitive

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*This comment applies to all the experiments with phytoplankton reviewed in this section, since the actual concentration of PCBs in the culture medium does not appear to have been measured in any of the studies.

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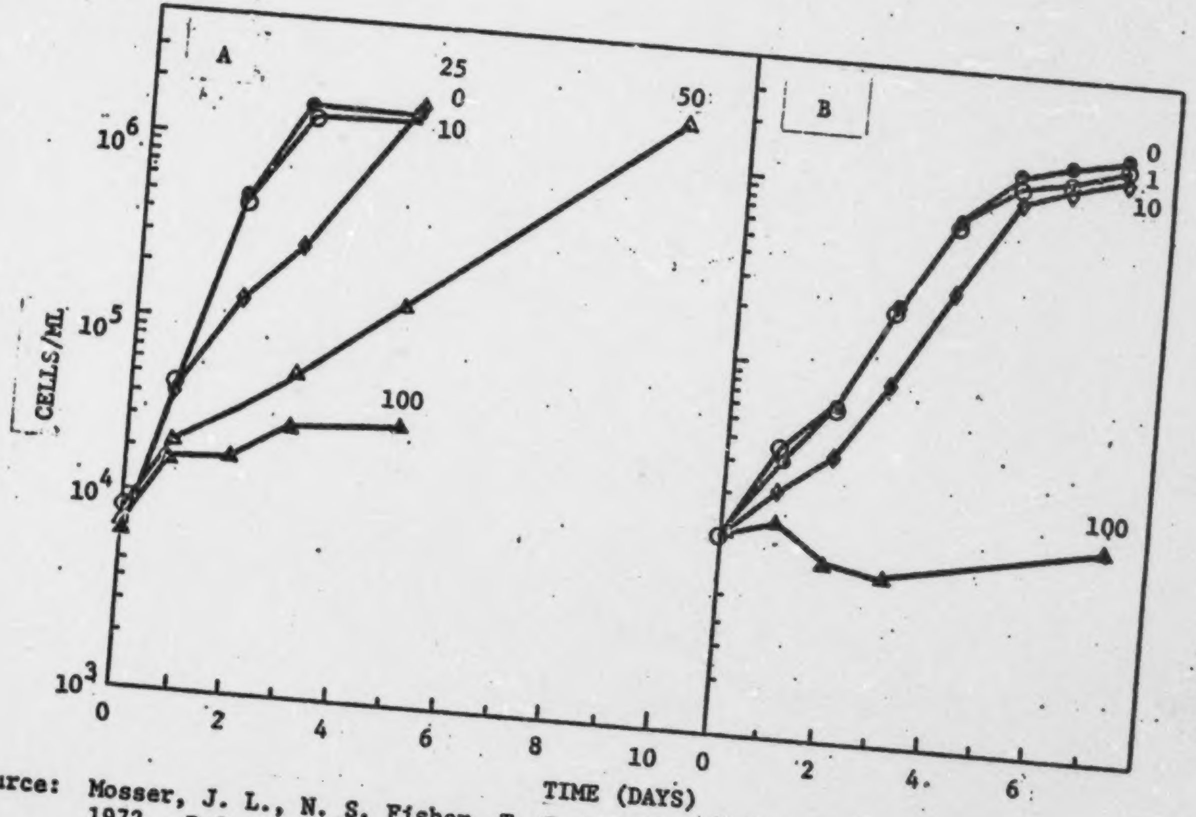


Source: Mosser, J. L., N. S. Fisher, T.-C. Teng, and C. F. Wurster, 1972. Polychlorinated biphenyls: toxicity to certain phytoplankters. Science 175(4018):191-192.

Effects of Aroclor 125

pseudonana

Table III.3.4 (from ref. 90)

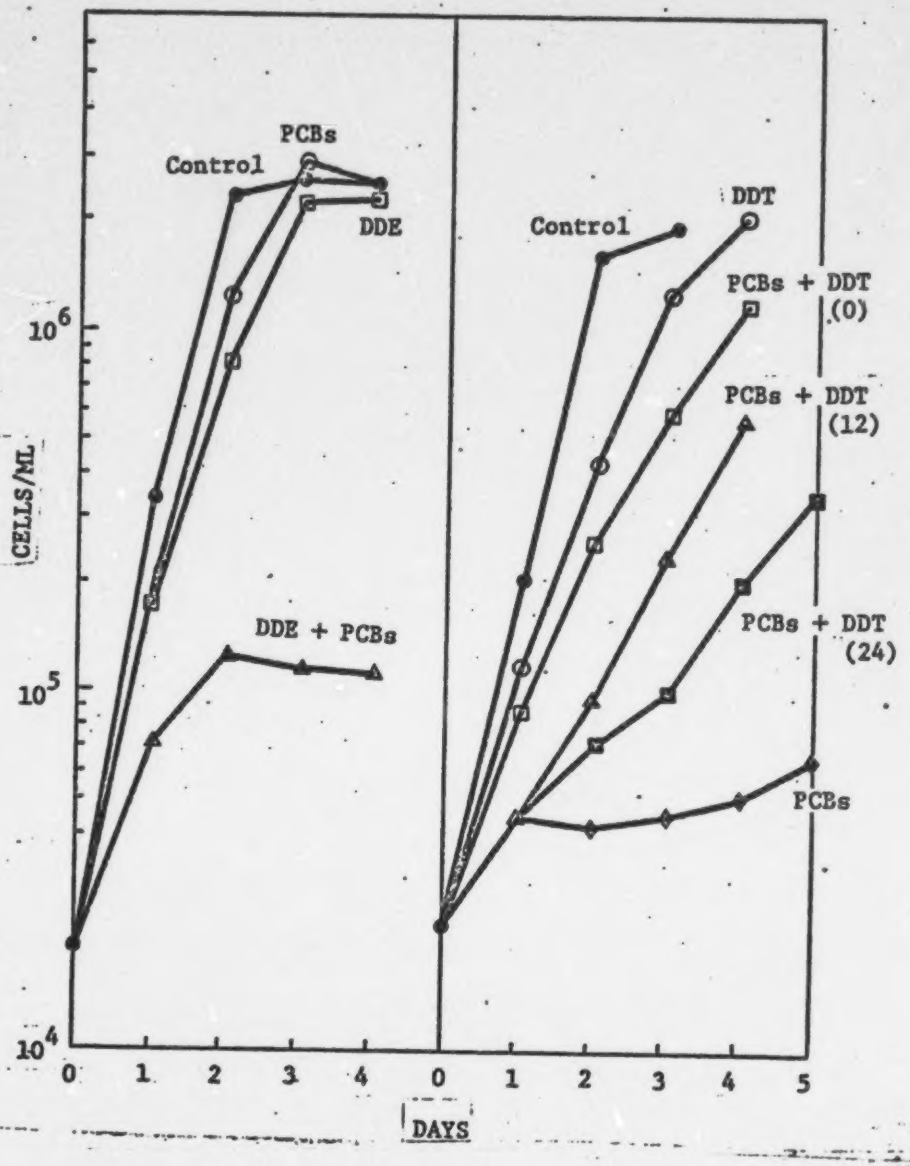
RATIOS OF MEAN CELL DENSITIES FROM 10ppb
AROCLOR 1254-TREATED CONTROL CULTURES AT FIVE SAMPLE TIMES

	HOURS AFTER INOCULATION				
	0	47.5	70	95	118
Thallassiosira pseudonana clones					
3H (estuarine)	1.00	0.694	0.365	0.676	0.976
7-15 (continental shelf)	1.00	0.544	0.368	0.520	0.844
13-1 (oceanic)	1.00	0.422	0.241	0.172	0.341
Fragilaria pinnata clones					
0-12 (estuarine)	1.00	0.824	0.909	0.932	0.590
13-3 (oceanic)	1.00	0.688	0.707	0.269	0.387
Bellerochia clones					
Say-7 (estuarine)	1.00	0.207	0.064	0.036	0.035
SD (oceanic)	1.00	0.074	0.020	0.013	0.005

than its counterpart from the estuary.

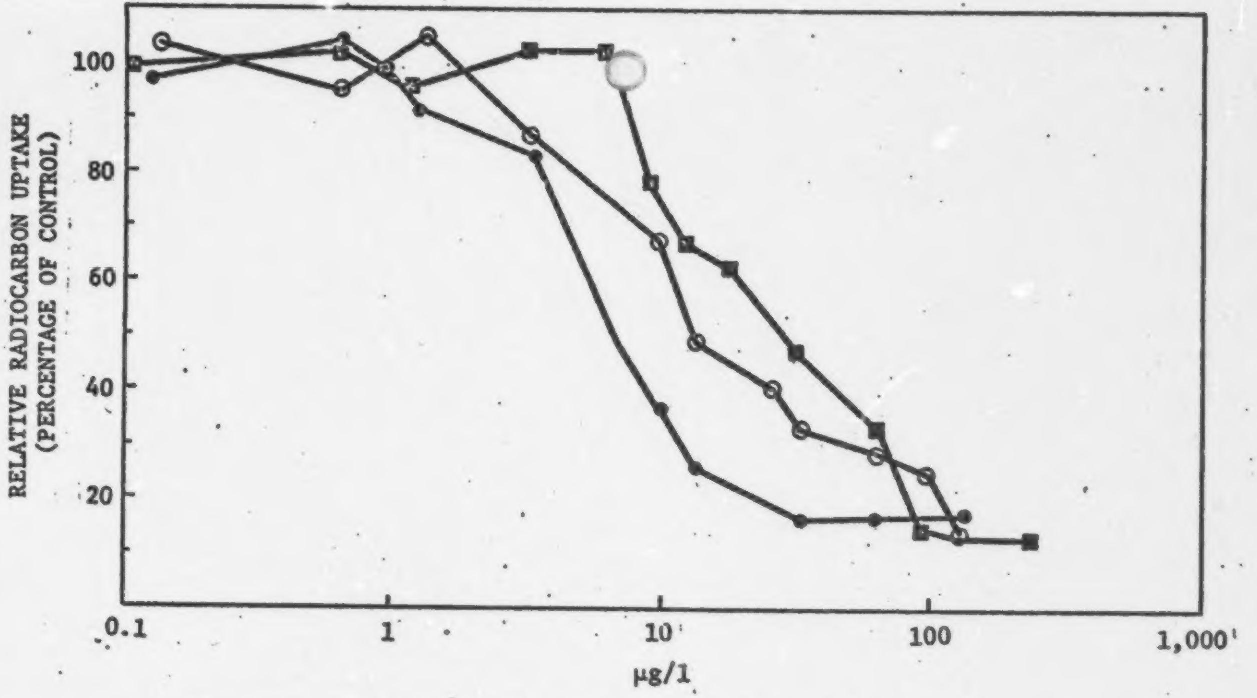
Synergistic effects occurred when DDE and Aroclor 1254 were used simultaneously with the diatom Thalassiosira pseudonana (90). At concentrations of 10 ppb Aroclor 1254 or 100 ppb DDE, minor growth inhibition occurred. However, used at the same concentrations simultaneously, there was substantial inhibition of growth, with still greater inhibition at higher concentrations (see Figure III.3.3). However, the effects of DDT were opposite to those of DDE. At 50 ppb, Aroclor 1254 almost stopped diatom growth. When 500 ppb DDT was added simultaneously with 50 ppb Aroclor 1254, growth was restored to two-thirds of the control (see Figure III.3.3). Lower DDT concentrations reversed Aroclor 1254 inhibition to a lesser extent. If DDT was added 12 or 24 hours after Aroclor 1242 inhibited diatom cultures, growth was again restored. Hence, co-precipitation of the inhibitor PCB by DDT in the medium was not the mechanism of reversal, but some intracellular reaction must be involved (90).

The uptake of carbon by natural plankton communities was affected by Aroclor 1242, Aroclor 1254, and 2,4'-dichlorobiphenyl (91). The plankton communities were dominated by the diatom genera Ceratulina, Chaetoceros, Biddulphia, Eupodiscus, Leptocylindrica, Melosira, Nitzschia, Rhizosolenia, and Thalassionema. Two dinoflagellate genera were also generally present: Peridinium and Ceratium. Sensitivity to Aroclor 1242 and 1254 was apparent at 1-2 ppb and to the dichlorobiphenyl at 7 ppb (see Figure III.3.4). In natural phytoplankton communities all stages of growth are represented through various phytoplankton species. Hence, while



Interactions among PCBs, DDE and DDT in Thallassiosira pseudonana.

Figure III.3.3 (from ref. 90



2,4'-dichlorobiphenyl (■); 'Aroclor 1242' (●) and 'Aroclor 1254' (○).

Adapted from: Moore, S. A., Jr. and R. C. Harriss. 1972. Effects of polychlorinated biphenyl on marine phytoplankton communities. Nature 240: 356-358.

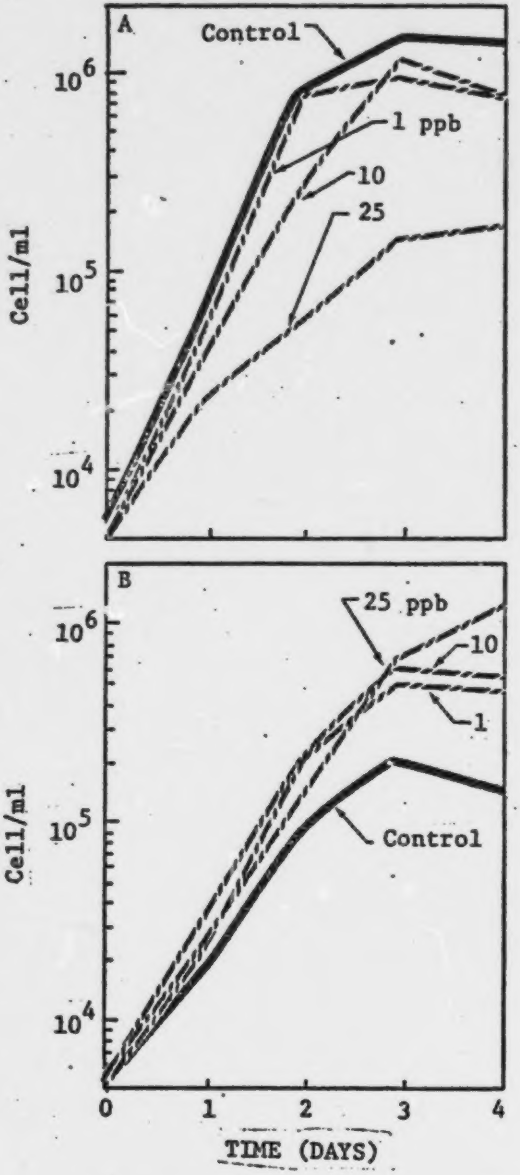
Effect of organochlorines on in situ radiocarbon uptake by marine phytoplankton communities.

Figure III.3.4 (from ref. 91)

overall photosynthesis diminished in the community exposed to PCBs, the precise species and/or active site cannot be identified. Nor were changes in species composition investigated.

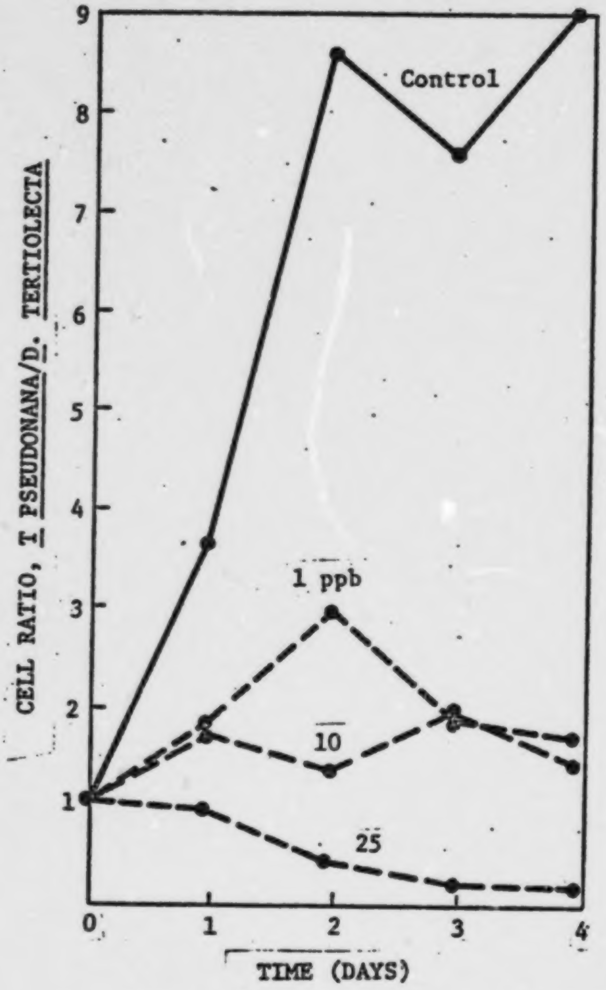
In a key experiment, Mosser _et al_. (88) tested the effects of PCBs on growth of a resistant species (the green alga _D. tertiolecta_) and a sensitive species (the diatom _T. pseudonana_) in a mixed culture. Although there were only slight effects on growth of either species at concentrations of 1 or even 10 ppb of Aroclor 1254 when cultured alone, there was a large change in the species ratio when the two species were exposed together to 1 ppb (Figures III.3.5 and III.3.6). Thus Aroclor 1254 significantly diminished the competitive success of the diatom and increased that of the alga, even at concentrations well below that required for detectable effects on either alone (88). Similar effects on competition between sensitive and resistant species were demonstrated at still lower PCB levels (0.1 ppb) by Fisher _et al_. (92). Figure III.3.7 shows the effect of 0.1 ppb Aroclor 1254 on _T. pseudonana_ in continuous culture with the resistant species _D. tertiolecta_. Table III.3.5 shows the effects of 0.1 ppb Aroclor 1254 on a natural phytoplankton community in continuous culture. Not only was the population of the sensitive _T. pseudonana_ markedly reduced, but the diversity of species in the culture was also decreased (92, Table 3).

Such changes in species composition are potentially the most important effects of PCBs on phytoplankton. Most species of zooplankton graze selectively, often choosing their food on the basis of size or shape. Studies on oysters, barnacles, clams,



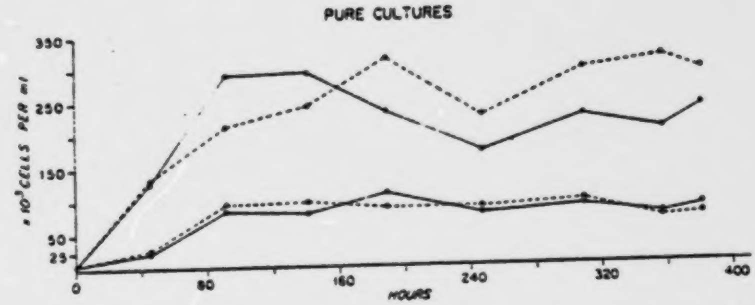
Growth of (A) Thallassiosira pseudonana and (B) Dunaliella tertiolecta in mixed cultures with Aroclor 1254 added.

Figure III.3.5 (from ref. 88)

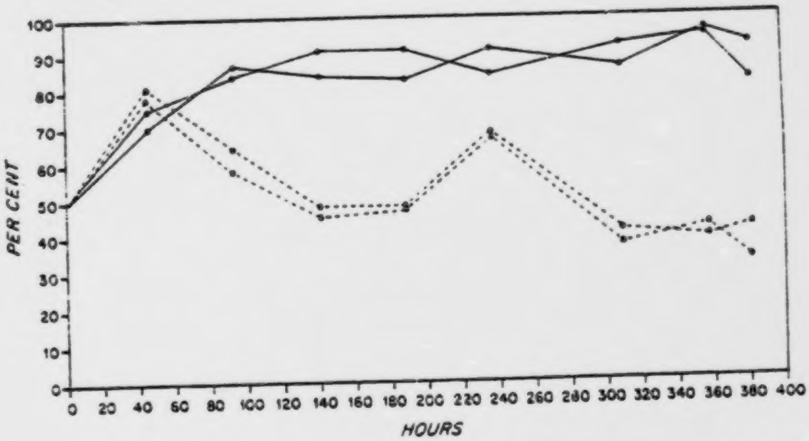


Species ratios in mixed cultures treated with Aroclor 1254.

Figure III.3.6 (from ref. 88)



Growth in continuous pure culture of *T. pseudonana* (•———•) and *D. tertiolecta* (○———○) with (- - -) or without (———) 0.1 ppb of PCB. Data points are means from duplicate cultures. A single classification analysis of variance [37] showed that treated and control culture values did not differ significantly for either species.



Continuous cultures of gnotobiotic communities containing *T. pseudonana* and *D. tertiolecta*, showing percent of total cell counts represented by *T. pseudonana* growing with (- - -) or without (——) 0.1 ppb of PCB. Duplicate values are shown.

Figure III.3.7 (from ref. 92)

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Table III.3.5 (from ref. 92)

Growth of the Three Dominant Species in Natural Phytoplankton Communities in Continuous Culture[a]

		T. pseudonana (X 10^3 cells/ml)		*Chaetoceros* sp. (X 10^3 cells/ml)		*S. costatum* (X 10^3 cells/ml)	
	Hours	**0 ppb**	**0.1 ppb**	**0 ppb**	**0.1 ppb**	**0 ppb**	**0.1 ppb**
A	0	4.0 ± 1	4.0 ± 0	5	4	3	1
	146	23.5 ± 0.5	2.5 ± 1.5	195	335	23	12
B	41	2.0 ± 0.5	2.3 ± 1.3	32	12	4	3
	184	11.5 ± 5.5	3.0 ± 2	469	378	7	7

[a] Initial and final cell densities are means from duplicate treated and control cultures. The dilution rate for experiment A was 33 ± 3% of the volume/24 hr; for experiment B was 47 ± 4% of the volume/24 hr.

and copepods have shown that the dietary requirements of herbivores are not satisfied by all species of algae (88). The replacement of sensitive diatoms by resistant green algae in marine phytoplankton communities could thus have profound effects on their consumers and hence on the distribution and abundance of many animal populations higher in the food web (83, 88). In experiments with single species, effects on growth have been demonstrated at PCB concentrations as low as 0.1 ppb (84, 86). The experiments reported in refs. 88 and 92 show that effects on species composition and hence on foodwebs are to be expected at still lower levels (83, 92).

Although most experiments with phytoplankton have been carried out with Aroclor 1254, Aroclor 1242 inhibited growth of Scenedesmus at a concentration of only 0.1 ppb (84), and in the experiment with a mixed culture conducted by Moore and Harriss (91), Aroclor 1242 was more active than Aroclor 1254 (Figure III.3.4). Hence tetra- and trichlorobiphenyls are probably at least as toxic to phytoplankton as higher chlorobiphenyls.

III.4. Effects on Aquatic Invertebrates

Effects of PCBs on aquatic invertebrates have recently been reviewed by Walker, Nebeker, and Hansen (73-75).

III.4.1. Effects on fresh-water organisms

Fresh-water crustaceans are very sensitive to low concentrations of PCBs. Table III.4.1 summarizes the results of static and continuous flow bioassays conducted with Daphnia magna using eight Aroclor mixtures (93). The mixtures appeared much more toxic in continuous-flow conditions due to continual replacement

CALCULATED 2- AND 3-WEEK LC50 AND 16% REPRODUCTIVE IMPAIRMENT VALUES FOR DAPHNIA MAGNA SUBJECTED TO AROCLOR 1248 AND AROCLOR 1254 IN CONTINUOUS-FLOW CONDITIONS AND EIGHT AROCLORS IN STATIC CONDITIONS AT 18 ±1 C

Aroclor (PCB)	3-wk LC50 (µg/liter)	Confidence limits (0.05)	Reproductive impairment (µg/liter)	
			50%	16%
		Static test conditions		
A-1221	180	158.0-205.0	125	89
A-1232	72	62.6- 82.8	66	53
A-1242	67	55.4- 81.0	63	48
A-1248	25	21.4- 29.2	24	16
A-1254	31	25.8- 37.2	28	18
A-1260	36	27.7- 46.8	33	22
A-1262	43	37.0- 49.9	41	24
A-1268	253	222.0-288.0	206	162
		Continuous-flow test conditions		
A-1248	2.6 (2-wk LC50)		2.1	1.0
A-1254	1.8 (2-wk LC50)		1.1	0.48
A-1254	1.3 (3-wk LC50)		1.3	1.0

Source: Nebeker, A.V. and F.A. Puglisi. 1974. Effect of Polychlorinated Biphenyls (PCBs) on Survival and Reproduction of *Daphnia*, *Gammarus*, and *Tanytarsus*. *Transactions of the American Fisheries Society* 103(4):722-728.

Table III.4.1 (from ref. 93)

of fresh PCBs. In static conditions much of the PCBs added to the test water was lost to the air, with some lost to bacteria, algae, waste materials, container surfaces, and animal tissues. The differences shown in Table III.4.1 between results in static and continuous-flow bioassays show how critical the test methodology can be for hydrophobic chemicals such as PCBs (74).

In the static tests with Daphnia magna, Aroclor 1248 was the most toxic of the eight mixtures tested; the 3-week LC_{50} was 25 μg/l. In the continuous-flow tests, Aroclor 1254 was more toxic, with a 3-week LC_{50} of 1.3 μg/l. No reproduction occurred at 3.8 μg/l, and reproduction was significantly impaired at concentrations below 1.0 μg/l (Table III.4.1).

The toxicity of Aroclor 1254 to Daphnia magna is significantly enhanced by the presence of DDT, although the effects of the two compounds are less than additive (94). In a static bioassay system, the two-week LC_{50}s for DDT and Aroclor 1254 to Daphnia were 0.67 ppb and 24.0 ppb, respectively. Addition of 12 ppb Aroclor increased the toxicity of DDT by approximately one-third, and addition of 0.5 ppb DDT doubled the toxicity of Aroclor 1254 (Table III.4.2).

The fresh-water scud Gammarus pseudolimnaeus was also studied in continuous-flow test systems to determine the effects of Aroclors 1242 and 1248 on survival and reproduction (93). Concentrations as low as 8.7 μg/l Aroclor 1242 and 5.1 μg/l Aroclor 1248 caused approximately 50 percent mortality in 60 days' exposure and seriously affected reproduction (Table III.4.3).

Table III.4.4 summarizes the results of static and continuous-flow tests with five other species. The glass shrimp Palaemonetes

Table III.4.2 (from ref. 94)

The concentrations (ppb) of p,p' DDT and Aroclor 1254 alone and in combination that affect survival (LC_{50}) and reproduction (EC_{50}) of *Daphnia magna* in a 14 day test.

Toxicant Combination	Acute Toxicity 14-day LC_{50}		Reproductive Inhibition EC_{50} (ppb)					
			Total Young		Average Brood Size		Percentage of Days Reproducing	
	mean	C.L.*	mean	C.L.	mean	C.L.	mean	C.L.
p,p' DDT only	0.67	(0.65- 0.69)	0.50	(0.48- 0.52)	0.61	(0.58- 0.64)	0.75	(0.71- 0.79)
DDT with 12 ppb Aroclor 1254	0.40	(0.38- 0.41)	0.30	(0.28- 0.32)	0.45	(0.43- 0.48)	0.55	(0.52- 0.58)
Aroclor 1254 with 0.75 ppb DDT	4.0	(3.80- 4.20)	1.8	(1.69- 1.91)	6.0	(5.82- 6.18)	9.0	(8.65- 9.36)
Aroclor 1254 with 0.50 ppb DDT	12.0	(11.54-12.48)	10.0	(9.17-10.90)	13.0	(11.92-14.17)	11.0	(10.09-11.99)
Aroclor 1254 with 0.30 ppb DDT	14.0	(13.46-14.56)	13.0	(12.26-13.78)	19.0	(18.27-19.76)	19.0	(17.92-20.14)
Aroclor 1254 only	24.0	(23.08-24.96)	19.0	(17.93-20.14)	23.0	(22.12-23.92)	25.0	(23.81-26.25)

*Confidence limits for $p = 0.05$.

Table III.4.3 (from ref. 93)

Survival and reproduction of *Gammarus pseudolimnaeus* after 2 months' exposure to Aroclor 1242 and 1248

Aroclor concentration (µg/1)	Survival of adults (percent)	Young per surviving adult
Aroclor 1242		
234.0	0	0
81.0	0	0
26.0	0	0
8.7	52	0
2.8	77	4
0.0	48	7
Aroclor 1248		
18.0	0	0
5.1	53	7
2.2	73	22
0.5	71	20
0.2	73	11
0.0	64	11

Table III.4.5 (from ref. 93)

Table 5. Effect of Aroclor 1254 on the growth and survival of the midge *Tanytarsus dissimilis*

Aroclor concentration (µg/1)	Number of mature larval cases (percent of control)	Number of pupal cases (percent of control)
33.0	0	0
9.0	0.2	0
3.5	7	7
1.2	35	18
0.4	52	55
0.0 (control)	100	100

Table III.4.4 (from ref. 95)

Aroclor and DDT toxicity to invertebrates.

Compound	Organism[1]	Bioassay type[2]	Exposure (days)	LC_{50} ($\mu g/l$)
Aroclor 1242	Crayfish	static	7	30
Aroclor 1254	Crayfish	static	7	100
Aroclor 1254	Crayfish	continuous-flow	7	80
DDT	Crayfish	static	4	100
Aroclor 1242	Scud	continuous-flow	4	10
Aroclor 1242	Scud	continuous-flow	10	5.0
Aroclor 1248	Scud	static	4	52
Aroclor 1254	Scud	static	4	2,400
DDT	Scud	static	4	3.2
DDT	Scud	continuous-flow	5	0.6
Aroclor 1254	Glass shrimp	continuous-flow	7	3.0
DDT	Glass shrimp	static	5	1.0
DDT	Glass shrimp	continuous-flow	5	1.3
Aroclor 1242	Dragonfly	static	7	800
Aroclor 1254	Dragonfly	static	7	1,000
Aroclor 1242	Damselfly	continuous-flow	4	400
Aroclor 1254	Damselfly	continuous-flow	4	200
DDT	Damselfly	static	4	56

[1] Crayfish (*Orconectes nais*), Scud (*Gammarus fasciatus*), Glass shrimp (*Palaemonetes kadiakensis*), Dragonfly (*Macromia sp.*), Damselfly (*Ischnura verticalis*).

[2] Temperature, 15.6°C; alkalinity, 35 ppm; pH, 7.1.

kadiakensis was very sensitive to Aroclor 1254, with a 7-day LC_{50} of 3 μg/l (95).

In a comparative toxicity test with five individual chlorobiphenyls under static conditions, Mayer et al. (96) found that 2,3,4'-trichloro-biphenyl was slightly more toxic to G. pseudolimnaeus than representative di-, tetra-, penta-, or hexachlorobiphenyls; the 96-hour LC_{50} valves were 70, 100-120, 110, 210 and 150 μg/l respectively. Under static test conditions, the toxicities of Aroclors 1016 and 1242 to D. magna were similar: no young survived after 3 weeks' exposure to 125 μg/l of either mixture (74, 97).

Aquatic insects also appear to be very sensitive to prolonged exposure to PCBs (74). The midge Tanytarsus dissimilis was tested through its full life cycle (93) and found to be affected at levels below 1 μg/l. Survival and growth of the midge, when tested with Aroclor 1254, were excellent in control chambers but were reduced by 50 percent at the lowest test concentration of 0.45 μg/l (Table III.4.5). At 1.2 μg/l, the numbers of larval cases were reduced to 35 percent of the control, and the numbers of pupal cases were reduced to 18 percent of the control. No adult emergence occurred above 3.5 μg/l, and abundant adult emergence did not occur above 3 μg/l, even though larvae were present. No pupal cases were constructed at 9 μg/l and no larval cases were formed at 33 μg/l A-1254. The calculated 3-week LC_{50} for A-1254 (50-percent reduction based on control as 100 percent) was 0.65 ug/l for larvae and 0.45 μg/l for pupae. Adult midges emerged at concentrations up to 9 μg/l Aroclor 1248. Larvae were present at 18 μg/l, but adult emergence did not occur. Abundant emergence did not occur above 5.1 μg/l (93).

Studies by Sanders and Chandler (98) have shown that larvae of the stonefly *Pteronarcys dorsata*, the dobsonfly *Corydalus cornutus*, and the phantom midge *Chaoborus punctipennis* survived concentrations of up to 2.8 μg/l for up to 21 days without significant mortality. However, in similar experiments, larvae of the mosquito *Culex tarsalis*, exposed to 1.5 μg/l Aroclor 1254, survived well and pupated, but many of the pupae were unable to metamorphose into the adult form. Control organisms pupated with success.

III.4.2 Effects on estuarine organisms

PCBs are acutely toxic to estuarine organisms (74,99-101). For Aroclors 1016, 1242 and 1254, the 48-hour or 96-hour LC_{50} values for brown shrimps *Penaeus aztecus*, pink shrimps *P. duorarum*, and grass shrimps *Palaemonetes pugio* fall into the range 9-32 μg/l (74,99,100). Exposure to 10 μg/l Aroclor 1016 for 96 hours caused 43% mortality in brown shrimps and 38% mortality in grass shrimps (100), whereas the same concentration of Aroclor 1254 caused no mortality in pink shrimps in the same period (102). The acute toxicities of Aroclors 1016 and 1254 to oysters *Crossostrea virginica* appeared to be similar (99,100).

Laboratory bioassays lasting longer than two weeks have demonstrated that acute bioassays underestimate the toxicities of Aroclors 1016 and 1254. Aroclor 1254 is toxic to commercially valuable penaeid shrimps and grass shrimps at concentrations as low as 0.9-1.4 μg/l (102-105; Tables III.4.6, III.4.7). Exposed shrimps are particularly sensitive to salinity stress (103) and apparently to viral disease (106,107). Aroclor 1254 also decreases

Table III.4.6 (from ref. 102)

RESULTS OF CHRONIC BIOASSAYS WITH AROCLOR 1254 AND THE PINK SHRIMP *PENAEUS DUORARUM* IN FLOWING WATER

Shrimp rostrum-telson length (cm)	Concentration[a] (ppb)	Average salinity (%)	Average temperature (°C)	No. of test individuals	Replicates	Days Exposed	Average mortality (%)	Level of Significance
2.5-3.8	Control	32	29	65	5	15	12	--
2.5-3.8	0.57	32	29	20	2	15	30	0.10[b]
2.5-3.8	0.94	32	29	45	3	15	51	0.005[b]
2.5-3.8	9.4	32	29	20	2	15	90	0.001[b]
2.5-3.8	19.0	32	29	20	2	15	100	0.001[b]
4.2-7.2	Control	29	28	25	1	32	4	--
4.2-7.2	2.4	29	28	20	1	17	65	0.001[c]
4.2-7.2	3.1	29	28	25	1	32	80	0.001[c]
6.6-9.0	Control	29	20	43	1	53	26	--
6.6-9.0	4.3	29	20	46	1	53	83	0.001[c]
7.6-8.5	Control	31	29	60	1	18	9	--
7.6-8.5	4.0	31	29	60	1	18	41	0.001[c]
9.5-12.5	Control	28	20	50	1	35	8	--
9.5-12.5	3.5	28	20	50	1	35	50	0.001[c]

-- No data
[a] Average of at least three determinations.
[b] Student's t-test.
[c] Chi-square.

Table III.4.7 (from ref. 104)

MORTALITY AND ACCUMULATION OF AROCLOR 1254 IN Palaemonetes pugio*

Test Conc. (μg/ℓ)	Days Exposed	Average Mortality (%)**	Body Conc. (mg/kg)	Concentration Factor
CONTROL	7	4(0 - 20)	0.1	-----
0.17	7	8(0 - 40)	1.3	7600
0.62	7	4(0 - 20)	5.4	8700
9.1	7	60(20 - 80)***	65.0	7100
CONTROL	16	25(0 - 50)	<0.1	-----
1.3	16	40(0 - 100)	18.0	14000
4.0	16	45(25 - 50)***	27.0	6700
12.5	16	55(50 - 75)***	46.0	3700

*All exposures were conducted in flowing seawater: salinity and temperature ranges were 22 to 28‰ and 17 to 28°C.

**5 replicates per concentration: at least 4 shrimp per replication.

***Significant at $P > 0.05$.

the growth rates of oysters at concentrations as low as 4 ug/l (111; Figure III.4.1).

III.4.3 Effects on a marine invertebrate community

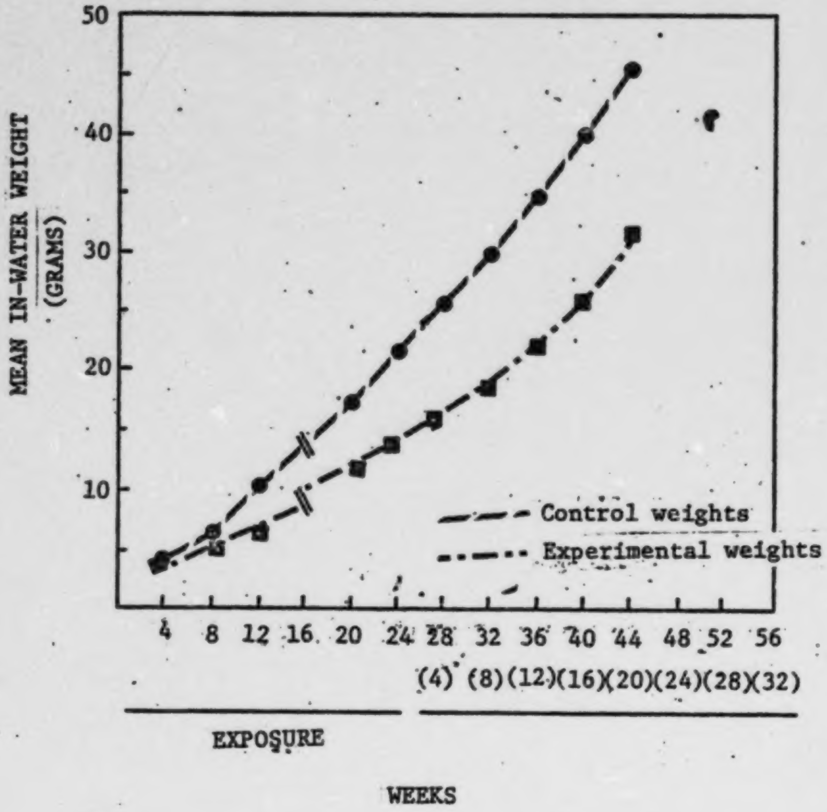
Hansen (109) showed that Aroclor 1254 affected the composition of communities of estuarine animals which developed from planktonic larvae in seawater flowing through aquaria in the laboratory. In control aquaria, the communities were dominated by arthropods, primarily the amphipod Corophium volutator. In aquaria receiving Aroclor 1254 at 1 or 10 µg/l, the number of arthropods decreased and the communities were dominated by tunicates (a group of animals characteristic of polluted waters). The numbers of amphipods, bryozoans, crabs, and molluscs were also reduced in these treated aquaria. These effects were manifested even in aquaria receiving only 0.1 µg/l of Aroclor 1254: the number and variety of molluscs were markedly reduced and the number of tunicates was increased (Table III.4.6). This experiment is important because it shows that PCBs can have marked effects on species composition and biological diversity at levels much lower than those required to demonstrate effects with single species.

III.5. Effects on Fish

Effects of PCBs on fish have recently been reviewed by Walker, Nebeker, and Hansen (73-75).

III.5.1. Lethal concentrations of PCBs to fish

Table III.5.1 summarizes measurements of the acute and chronic toxicities of eight Aroclor mixtures to four species of fish (73, citing data from refs. 95, 96). As in the case of the aquatic invertebrates, PCBs do not appear very toxic in short-term static



Change in oyster weight after exposure to 5 ppb Aroclor 1254 for 24 weeks.

Figure III.4.1 (from ref. 108)

Table III.4.6 (from ref. 109)

Species and total number of animals collected from the effluents of 10 control aquaria and 10 aquaria contaminated for four months with 0.1, 1 or 10 μg/l Aroclor 1254.

Taxon	Control	0.1 μg/l	1 μg/l	10 μg/l
Annelida				
Eupomatus dianthus	1	1	0	0
E. protulicola	0	1	1	0
Neanthes succinea	1	2	2	0
Total	2	4	3	0
Arthropoda				
Balanus sp.	10	2	2	1
Caprella sp.	0	8	4	0
Clibanaris tricolor	0	1	0	0
Corophium volutator	7	15	32	1
Eurypanopeus depresus	5	2	5	0
Neopanope texana	10	6	3	0
Pagurus longicarpus	1	0	0	0
Pinniza chaetopterana	1	0	0	0
Upogebia affinis	1	1	0	0
Decapod zoea, unident. sp.	0	0	1	0
Portunidae, unident. sp.	2	1	0	0
Pycnogonidae, unident. sp.	0	0	3	0
Total	37	36	50	2
Chordata				
Bostrichobranchus pilularis	3	0	0	0
Branchiostoma caribaeum	1	0	1	0
Molgula manhattensis	72	103	304	35
Total	76	103	305	35
Coelenterata				
Leptomedusae*, unident. sp.	1	1	1	1
Echinodermata				
Hemipholis elongata	1	0	3	0
Ectoprocta				
*Membranipora tenuis**	1	1	1	0
Mollusca				
Anadara ovalis	1	0	0	0
A. transversa	12	0	4	1
Bittium alternata	0	1	0	0
B. varium	3	0	1	0
Crassostrea virginica	2	0	1	1
Doridella obscura	12	1	2	1
Laevicardium mortoni	1	0	0	0
Mitrella lunata	2	0	2	2
Musculus lateralis	1	0	1	0
Nassarius albus	2	0	0	0
Tagelus divisus	1	2	0	0
Eolidacea, unident. sp.	1	1	1	3
Total	38	5	12	8
Totals: Animals	156	150	375	47
Species	27	18	20	10

* Colonies: Counted as one animal.

Table III.5.1 Toxicity of PCBs to fish (refs. 73, 95, 96)

PCB (Aroclor)	Fish	Temp (°C)	Bioassay method[1]	LC$_{50}$ value in µg/l at exposure time of						
				4d	5d	10d	15d	20d	25d	30d
1221	Cutthroat trout	8.9	A	1170	--	--	--	--	--	--
1232	Cutthroat trout	8.9	A	2500	--	--	--	--	--	--
1242	Cutthroat trout	8.9	A	4530	--	--	--	--	--	--
	Rainbow trout	17.0	C	--	67	48	18	10	12	--
	Bluegill	20.0	C	--	154	72	54	--	--	--
	Bluegill	17.0	C	--	--	--	164	125	120	84
	Channel catfish	20.0	C	--	--	174	107	--	--	--
	Channel catfish	17.0	C	--	--	--	219	150	132	87
1248	Cutthroat trout	8.9	A	5750	--	--	--	--	--	--
	Rainbow trout	17.0	C	--	54	38	16	6.4	3.4	--
	Bluegill	18.3	A	278	--	--	--	--	--	--
	Bluegill	20.0	C	--	307	160	76	10	--	--
	Bluegill	17	C	--	136	115	111	106	100	78
	Channel catfish	18.3	A	6000	--	--	--	--	--	--
	Channel catfish	27.0	C	--	--	94	57	--	--	--
	Channel catfish	20.0	C	--	--	225	127	--	--	--
	Channel catfish	17.0	C	--	--	121	121	115	104	75
1254	Cutthroat trout	8.9	A	42500	--	--	--	--	--	--
	Rainbow trout	20.0	C	--	156	8	--	--	--	--
	Rainbow trout	17.0	C	--	--	160	64	39	27	--
	Bluegill	18.3	A	2740	--	--	--	--	--	--
	Bluegill	20.0	C	--	--	443	204	135	54	--
	Bluegill	17.0	C	--	--	--	303	260	239	177
	Channel catfish	18.3	A	12000	--	--	--	--	--	--
	Channel catfish	20.0	C	--	--	--	741	300	113	--
	Channel catfish	17.0	C	--	--	303	286	293	181	139

Table III.5.1 (continued)

1260	Cutthroat trout	8.9	A	60900	—	—	—	—	—	—
	Rainbow trout	20.0	C	—	156	5	—	—	—	—
	Rainbow trout	17.0	C	—	—	326	143	78	49	51
	Bluegill	20.0	C	—	—	—	—	245	212	151
	Bluegill	17.0	C	—	—	—	—	—	—	400
	Channel catfish	20.0	C	—	—	—	—	296	166	137
	Channel catfish	17.0	C	—	—	535	482	512	465	433
1262	Cutthroat trout	8.9	A	50000	—	—	—	—	—	—
1268	Cutthroat trout	8.9	A	50000	—	—	—	—	—	—

1/ A= Acute toxicity by static test procedure
C= Chronic toxicity by flow-through procedure

bioassays, but are lethal at much lower concentrations on prolonged exposures. The rainbow trout was the most sensitive of the four species listed in Table III.5.1, being killed by levels as low as 3.4 µg/l Aroclor 1248 in 25 days, or 5 µg/l Aroclor 1260 in 10 days. Aroclor 1248 was generally the most toxic mixture tested in prolonged exposures.

In a separate series of tests under static conditions, the 96-hour LC_{50} for Aroclor 1016 was determined to be 650 µg/l for bluegills and 750 µg/l for channel catfish at 18°C; that for Aroclor 1221 was determined to be 230 ppm for bluegills and 3340 µg/l for channel catfish (110). These figures suggest that the acute toxicities of Aroclors 1016 and 1221 are comparable with or greater than those of Aroclor 1248 (Table III.5.1).

In chronic studies with estuarine fish, Aroclor 1016 appeared somewhat less toxic than Aroclor 1254. Spot and pinfish died when exposed for 14-45 days to 5 µg/l of Aroclor 1254 (111), whereas pinfish were only slightly affected by exposure for 42 days to 7 µg/l of Aroclor 1016 and their mortality was not significantly increased until the measured concentrations of Aroclor 1016 reached 13 µg/l (100). The longnose killifish is the most sensitive species tested, being killed by prolonged exposure to 1 µg/l of Aroclor 1254 (105).

Other recent work conducted by Mayer and Johnson has suggested that the toxicity of Aroclor 1016 to fresh-water fish is generally similar to that of Aroclor 1242 (75). Bluegills had a 96-hour LC_{50} of 48-260 µg/l Aroclor 1016; rainbow trout, 135 µg/l; Atlantic salmon, 134 µg/l; and yellow perch, 185 µg/l. On longer exposures, rainbow trout had a 10-day LC_{50} of 39 µg/l for Aroclor

1242 and a 17-day LC_{50} of 49 μg/l for Aroclor 1016. Bluegills had a 15-day LC_{50} of 54 μg/l for Aroclor 1242 and a 35-day LC_{50} for Aroclor 1016 (75).

All of the LC_{50} values decreased with higher temperatures and longer exposure to PCBs (Table III.5.1). An increase in temperature from 20° to 27°C approximately doubled the toxicity of Aroclor 1248.

Recent studies have shown that young fish are more sensitive than older fish (75, 112). Ninety-six-hour LC_{50} values for newly hatched fathead minnows were 15 μg/l for Aroclor 1242 and 7.7 μg/l for Aroclor 1254. After 60 days, half were dead at 8.8 μg/l Aroclor 1242 and 4.6 μg/l Aroclor 1254. Newly-hatched young were the most sensitive life stage. Growth of young fathead minnows was also affected at 2.2 μg/l Aroclor 1248 and none survived above 5.1 μg/l after 30 days. Young flagfish did not survive at concentrations of Aroclor 1248 above 5.1 μg/l and did not grow well above 2.2 μg/l (112).

III.5.2. Effects on fish reproduction

Two 9-month continuous-flow bioassay tests were conducted with fathead minnows to determine the effects of Aroclor 1242 and Aroclor 1254 on survival and reproduction (112). Concentrations of Aroclor 1242 above 10 μg/l were lethal to newly-hatched fry. Reproduction occurred and good egg hatching took place at and below concentrations of 5.4 μg/l, but the number of eggs produced was reduced at all concentrations tested down to 0.9 μg/l (Table III.5.2). Aroclor 1254 appeared more toxic than Aroclor 1242, as fathead minnows did not survive and reproduce at concentrations above 1.8 μg/l (Table III.5.2). Spawning was signifi-

Table III.5.2 (from ref. 112)

Table 2. Spawning and egg production of fathead minnows exposed to Aroclor 1242 and 1254

Aroclor concentration (μg/l)	Number of spawnings per female	Number of eggs per spawning	Number of eggs per female	Percent eggs hatched
Aroclor 1242				
51.0	0	0	0	0
15.0	0	0	0	0
5.4	2.5	30	151	81
2.9	3.9	63	283	38
0.9	1.3	28	35	84
0.0	4.6	90	442	53
Aroclor 1254				
15.0	0	0	0	0
4.6	0	0	0	0
1.8	1.0	63	106	79
0.5	5.2	105	556	63
0.2	3.0	64	221	55
0.0	2.4	104	253	75

cantly reduced at 1.8 µg/l; reproduction was good at 0.5 µg/l and lower concentrations. In chronic tests with Aroclors 1248 and 1260, fathead minnows were able to reproduce at concentrations which were acutely toxic to the larvae. Reproduction occurred at and below concentrations of 3 µg/l Aroclor 1248 and 2.1 µg/l Aroclor 1260, but mortality of the newly hatched larvae led to a 20 percent reduction in the population in the second generation at concentrations as low as 0.4 µg/l (113).

Aroclor 1254 also affects reproduction of the sheepshead minnow, an estuarine fish (114, 115). Adult fish exposed for 4 weeks to 0.1 µg/l appeared to be unaffected, but when eggs from these fish were fertilized and placed in PCB-free water, the survival of fry was diminished. Mortality was observed also in fry from eggs that contained more than 5 ppm of PCBs and increased as the PCB content of the eggs increased (75, 114, 115). However, sheepshead minnows exposed to Aroclor 1016 at levels from 0·3 to 3·0 µg/l of Aroclor 1016 reproduced successfully: although the eggs contained 3-77 ppm of PCBs, fry hatched from them appeared to be unaffected (116). This is one of the few examples in which Aroclor 1016 appears to have been significantly less toxic than other mixtures.

In tests with rainbow trout, fry from eggs containing 2·7 ppm Aroclor 1242 together with 0.09 ppm DDT suffered a 75 percent mortality in the 30 days after hatching. In five other groups of eggs with still lower residues, 10-28 percent mortality was noted within the first 30 days. About 60-70 percent of the fry that survived were deformed (117, 73). Swedish investigators have reported a statistically significant association between hatching failure in eggs of Atlantic salmon and PCB residues. Concentrations in the range 0·4 to 1·9 ppm, wet weight (7·7-34 ppm, lipid weight) were associated with mortalities between 16 and 100 percent (118).

Coho salmon eggs hatched two to five days premature when exposed to Aroclor 1254 (119; see Table III.5.3). Yolk-sac utilization was considerably reduced in the exposed eggs. Early hatching was preceded by changes in the egg surface, possibly decreasing the permeability of the chorion to oxygen and thus inducing early hatching (119). Survival following hatching was inversely related to the exposure concentration and time. Early hatching and reduced survival were noted even at the lowest concentration tested, 5 µg/l (Table III.5.3).

In contrast to these reported effects of low levels of PCBs on reproduction in rainbow trout and salmon, no adverse effects were observed on survival, growth, or reproduction of brook trout exposed for 71 weeks to 0·94 µg/l and lower concentrations of Aroclor 1254. Survival and growth of alevins from exposed parents were also unaffected (75, 120).

III.5.3. Other sublethal effects on fish

Inclusion of Aroclor 1254 in fish food at a level of 0·45 ppm resulted in a 52 percent increase in thyroid activity in coho salmon (Figure III.5.1). The lowest detectable stimulation of thyroid activity occurred in salmon ingesting a dose no more than one-thousandth of that required to cause mortality (73, 96). Similar results were obtained in channel catfish exposed to 2·4 ppm

Table III.5.3 (from ref. 119)

Table 6.41 INCUBATION TIMES AND HATCHABILITIES OF COHO SALMON (Oncorhynchus kisutch) EGGS, AND SURVIVAL AND SIZES OF ALEVINS 4 WEEKS AFTER HATCHING, AFTER EXPOSURE TO AROCLOR 1254 FOR 6 AND 2 WEEKS AT 12-14C

	Incubation			Mean alevin size	
PCB (ppb)	Time (degree-days)	Hatchability (%)	Alevin Survival (%)	Length (mm)	Weight (g)
	Exposure for 6 weeks (2 before and 4 after hatching)				
0	511	95.5	91.0	36.0	0.30
5	485	88.0	75.5	34.7	0.30
10	485	78.5	64.0	34.4	0.30
15	-	38.0[1]	24.0	31.9 (12)[2]	0.27
25	459	96.5	6.8	29.8 (4)	0.28
55	462	63.0	6.5	28.3 (3)	0.24
	Exposure for 2 weeks (to 2 days before hatching)				
0	503	93.0	93.0	35.3	0.30
5	475	95.5	69.5	35.2	0.30
10	490	90.0	88.0	34.7	0.30
15	490	91.5	83.5	33.3	0.26
25	481	97.5	74.5	34.5	0.29
55	488	66.0	39.5	33.7 (15)	0.26

[1] A fungus caused high mortality.

[2] Numerals in parentheses are numbers measured; 20 were used in each of other tests.

of Aroclor 1254; effects were smaller with other Aroclors (Figure III.5.2). These sublethal effects were associated with whole-body residues of PCB in the range 1-5 ppm (Figures III.5.1, III.5.2). Mayer et al. also found that incorporation of Aroclor 1248 into the diets of lake trout at concentrations in the range 0·2 - 6 ppm suppressed growth and serum cortisol levels, but stimulated thyroid activity during the first 160 days of exposure (73, 96).

Walker (73) also reported unpublished experiments in which lake trout exposed to PCBs in the diet developed lesions in the gills and liver during a 6 - 9 month exposure period. Nonspecific degeneration of liver parenchyma and cytoplasmic vacuolation of liver cells were reported in addition to pleomorphism. Spot exposed for two weeks or longer to 5 µg/l of Aroclor 1254 showed fatty changes in their livers, characterized by the presence of large vacuoles within hepatocytes and disorientation of liver cord distribution (105). Affected fish usually developed ragged fins and lesions on the body (111).

Pinfish sublethally poisoned by exposure for 14-45 days to 5 µg/l of Aroclor 1254 similarly developed fungus-like lesions on the body, especially around the mouth, where hemorrhaging also occurred (111). Pinfish similarly exposed to 32 µg/l of Aroclor 1016 suffered progressive erosion of scales, skin and finally, flesh in front of the dorsal fin. These fish also showed several changes in liver and pancreatic tissues. Normal liver cord orientation was altered and severe vacuolation developed in the pancreatic exocrine tissue surrounding the portal veins (100).

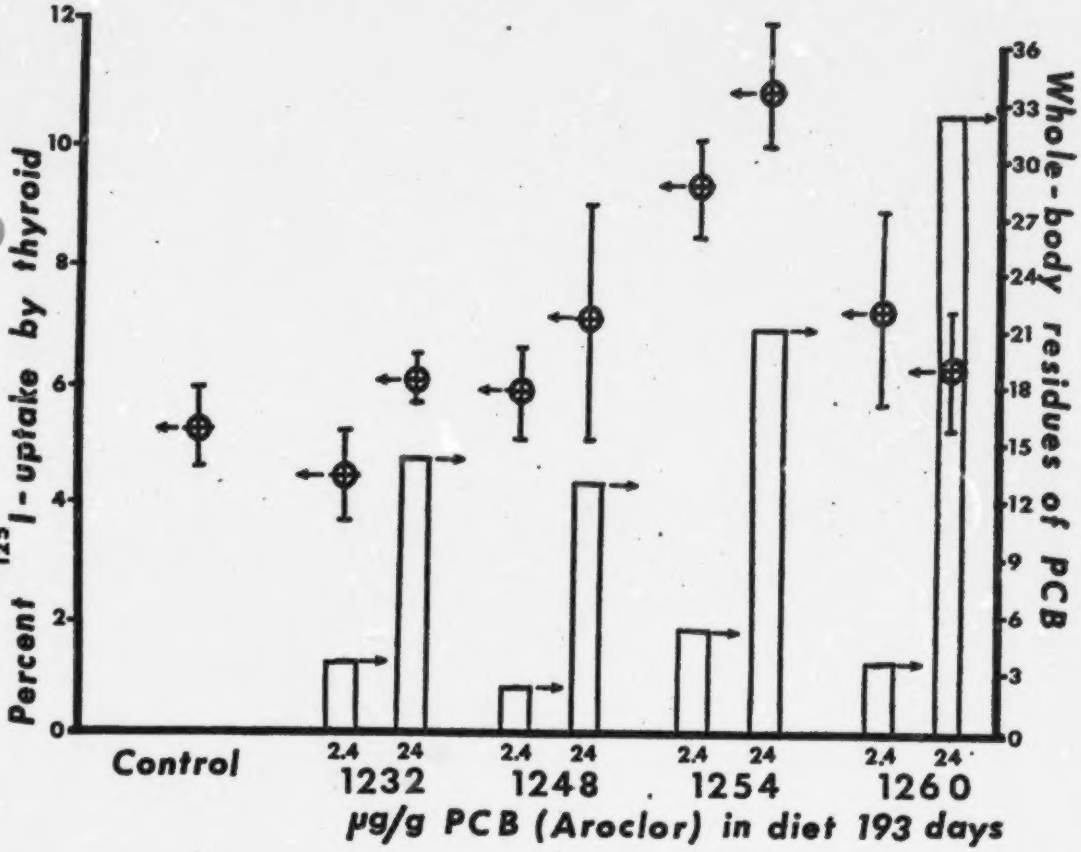


Figure 3. Whole-body residues and thyroidal uptake of ^{125} I in channel catfish fed diets containing Aroclors 1232, 1248, 1253, and 1260 for 193 days (ref. 40).

Figure III.5.1 (from ref. 73)

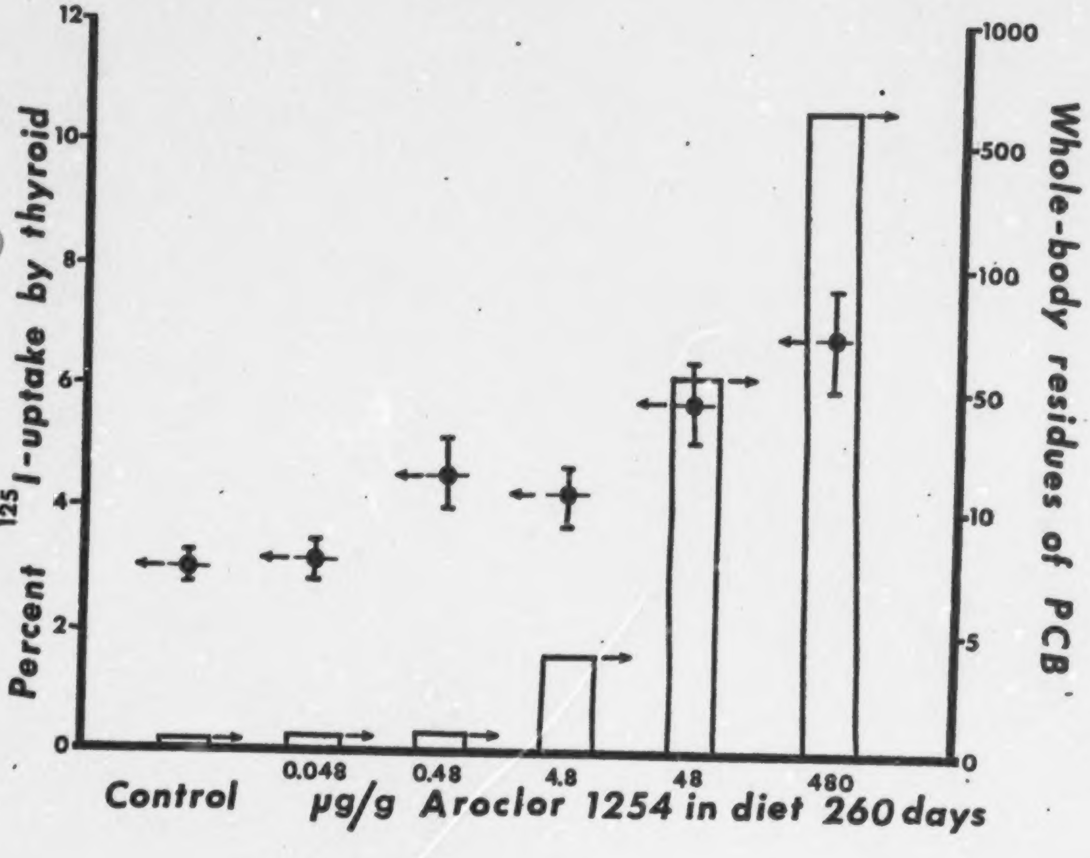


Figure 2. Whole-body residues and thyroidal uptake of ^{125}I in coho salmon fed diets containing Aroclor 1254 for 260 days (ref. 40).

Figure III.5.2 (from ref. 73)

"Fin rot syndrome" has been induced in the laboratory in spot exposed to 3-5 µg/l of Aroclor 1254 (106). The syndrome appears identical to that observed in wild fish (croakers and spot) which died under conditions of warm weather and oxygen depletion in Escambia Bay, Florida (106).

Growth of juvenile coho salmon was significantly reduced (from 1·0 percent of body weight per day to 0·7 percent) when they were fed a diet containing 10 ppm of a mixture of tetra- and hexa-chlorobiphenyls for 165 days (121).

Johansson et al. (122) found that sublethal doses of Clophen A 50 caused metabolic disturbances in brown trout (Salmo trutta). The experimental trout were given two doses (intramuscular injection or capsules directly into the stomach) of 5 mg/kg body weight on days one and five. Analyses were made on day 43 and, after 116 days starvation, on day 203. While the PCB-treated trout seemed to be in good condition after both 43 and 203 days, metabolic changes had taken place. Among the differences on day 43, the treated trout showed anemia, hyperglycemia and altered cholesterol metabolism. After starvation and refeeding, the PCB-treated trout differed from the controls by increased body-weight, a more pronounced decrease in liver triglycerides and muscle glycogen, and no hyperglycemic response.

III.5.4 Effects on ATPase and Osmoregulation

The effect of polychlorinated biphenyls on the ATPase enzyme system was examined in tissues of the bluegill sunfish (Lepomis machrochirus) by Yap et al. (123). ATPase activity was determined in homogenized tissue fractions that contained mitochondria plus nerve endings. Three Aroclors, 1221, 1254, and

1268, inhibited MG^{2+} and Na^+-K^+ ATPases in concentrations less than 1 ppm, with the most pronounced inhibition on Mg^{2+} ATPase of muscle (see Table III.5.4) (123). Cutkomp _et al_. (124) also found the maximum effects _in vitro_ were on oligomycin-insensitive* Mg^{2+} ATPase, with greatest inhibition in muscle. The effectiveness of Aroclor 1242 on oligomycin-insensitive and oligomycin-sensitive Mg^{2+} ATPase from bluegill muscle was compared at 50 percent inhibition, with values of 0.6 ppm and 2.0 ppm, respectively (124).

In general, the PCBs do not reveal any obvious relationships between chlorination and inhibitory effects. The action of Aroclor 1268 on Mg^{2+} ATPase from different tissues ranged from inhibition (muscle) to activation (brain, liver, and kidney), dependent upon concentration (124, 125). Aroclor 1242 inhibited Na^+-K^+ ATPase from fish brain homogenates but about five times the concentration was required as compared to inhibition of mitochondrial Mg^{2+} ATPase. Mitochondrial Mg^{2+} ATPase was more sensitive to Aroclor 1242 _in vivo_ than non-mitochondrial Mg^{2+} ATPase, the reverse of _in vitro_ findings with fathead minnows (124). Thus, although results are more accurate _in vitro_ than _in vivo_, both approaches seem desirable. Of the Aroclors tested, those in the intermediate range of chlorination appeared to have the greatest inhibitory effect on the ATPase system, especially Aroclor 1242. Data on inhibition of ATPase from brain, liver and kidney of fathead minnows (_Pimephales promelas_) are given in Tables III.5.5 and III.5.6 (126). Koch _et al_. (126) feel that there is sufficient evidence to indicate ATPase inhibition may be the

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*Oligomycin-insensitive--non-mitrochondrial.

Table III.5.4 (from ref. 123)

INHIBITION OF mg^{2+} ATPase and Na^+-K^+ ATPase FROM TISSUES OF BLUEGILL FISH

Concentration	Percentage Inhibition							
(p.p.m.)	'Aroclor 1221'		'Aroclor 1254'		'Aroclor 1268'		'Aroclor 5460'	
	Mg^{2+}	Na^+-K^+	Mg^{2+}	Na^+-K^+	Mg^{2+}	Na^{2+}-K^+	Mg^{2+}	Na^+-K^+
Brain								
0.03	0.3	4.6	4.2	16.9	0.4	29.4	2.3	26.9
0.33	8.4	10.5	0.7	19.1	+1.5	27.6	2.9	26.3
3.33	7.2	20.4	27.7	41.5	+14.0	43.6	2.5	38.9
10.00	36.0	46.4	57.9	45.5	8.3	39.4	11.2	42.7
Kidney								
0.33	7.4	15.1	1.3	35.2	0.0	25.4	0.0	22.3
3.33	5.0	24.3	33.4	45.9	+7.9	40.1	4.5	25.2
10.00	42.8	43.3	60.1	51.7	+4.8	48.0	+1.4	33.8
Liver								
0.03	2.2		3.9		—		—	
0.33	2.6		19.4		+5.3		6.6	
3.33	2.9		24.3		+5.1		9.3	
10.00	35.8		53.0		+2.4		15.8	
16.70	45.9		74.1		6.2		—	
Muscle								
0.03	18.7		20.6		37.2		31.9	
0.33	29.9		30.6		47.1		42.9	
3.33	65.4		73.1		60.3		—	
10.00	80.4		84.3		67.5		—	

Table III.5.5 (from ref. 126)

INHIBITION OF ACTIVITY OF ATPases BY A 4 MONTH EXPOSURE TO AROCLOR 1242 IN FAT HEAD MINNOW TISSUES

Concentration	Percent Inhibition*								
(ppb)		Brain			Kidney			Liver	
		Oligomycin**			Oligomycin			Oligomycin	
	Na^+ -K^+	Sens.	Insens.	Na^+ -K^+	Sens.	Insens.	Na^+ -K^+	Sens.	Insens.
		Mg^{2+}	Mg^{2+}		Mg^{2+}	Mg^{2+}		Mg^{2+}	Mg^{2+}
0.93	21.1	28.1	15.0	20.5	56.0	22.7	+41.6	0.8	7.9
2.8	4.9	53.2	2.3	36.2	75.4	20.7	+40.7	40.5	3.7
8.3	2.9	43.6	+0.7	18.3	45.2	35.0	42.4	40.7	15.5
Control***	34.3	5.2	12.6	39.3	9.0	18.4	3.6	7.6	8.2
Sp. Act.	±1.8	±1.1	±0.45	±2.02	±1.85	±1.35	±0.61	±1.6	±1.9
±S.E.									

* (+) Values represent increased enzyme activity.

** Sens. (sensitive) Insens. (insensitive).

*** Specific activity calculated as μmoles Pi mg^{-1} protein hr^{-1}. Each value represents the mean from three separate determinations.

Table III.5.6 (from ref. 126)

INHIBITION OF ACTIVITY OF ATPases BY A 4 MONTH EXPOSURE TO AROCLOR 1254 IN FAT HEAD MINNOW TISSUES

Concentration	Percent Inhibition*								
(ppb)	Brain			Kidney			Liver		
		Oligomycin**			Oligomycin			Oligomycin	
	Na^+ -K^+	Sens. Mg^{2+}	Insens. Mg^{2+}	Na^+ -K^+	Sens. Mg^{2+}	Insens. Mg^{2+}	Na^+ -K^+	Sens. Mg^{2+}	Insens. Mg^{2+}
0.31	+20.3	31.3	+21.8	+20.0	+53.7	+14.3	--	--	--
0.93	+11.2	35.2	+ 9.8	14.3	+ 7.3	+ 4.9	+20.8	+37.8	+11.4
2.8	+ 3.2	35.8	+ 5.5	5.1	3.3	+22.5	1.0	20.3	20.8
8.3	5.2	7.5	2.2	30.7	+ 4.2	+31.6	4.4	30.7	+ 2.2
Control***	32.6	5.2	12.5	31.4	7.9	12.6	5.9	7.9	9.5
Sp. Act.	±1.95	±0.34	±0.68	±2.45	±2.05	±3.85	±2.0	±2.31	±3.61
±S.E.									

* (+) Values represent enzyme activation.

** Sens. (sensitivity) Insens. (insensitive).

*** Specific activity calculated as μ moles Pi mg^{-1} protein hr^{-1}. Each value represents the mean from three separate determinations.

specific action of PCB in fish tissues. While kidney tissue appeared most sensitive to Aroclor 1242, both liver and kidney had undergone considerable degradation by Aroclors 1242 and 1254 when observed during dissection (126).

Aroclor 1221 affected the ability of the killifish to osmoregulate, since both osmolality and Na^+ concentration increased toward that of sea water (127). Since Na^+-K^+ ATPase is believed to be involved in the process of sodium transport across cell membranes and to function in osmoregulation in marine teleosts, Aroclor 1221 probably acts to disrupt the Na^+-K^+ ATPase system. Serum osmolality and K^+ concentrations were increased well above normal in fish blood sampled before the fish died (127).

III.5.5 Effects of PCDFs on fish

Zitko and Choi (128) fed a diet containing about 20 ppm of PCDFs (2.7 ug/g di-, 5.7 ug/g tri-,2.8 ug/g tetra- , and 9.1 ug/g octa-chlorodibenzofuran) to juvenile Atlantic salmon. This level of dietary exposure proved only moderately toxic, the median time to death being 120 days. For comparison, coho salmon died after 260 days when fed a diet containing about 100 ppm of PCBs (14.5 mg/kg body weight per day of Aroclor 1254) (96, 121). This indicates that PCDFs are somewhat more toxic than PCBs to fish, but not dramatically so as in the case of birds and mammals (see below).

III.6 Effects on birds

Effects of PCBs on birds were reviewed in 1972 by Vos (129) and most recently by Standell (130).

III.6.1 Lethal concentrations in diet: subacute and semichronic studies

The subacute toxicity of six Aroclor mixtures was studied in 2-week-old chicks of four species of wild birds (mallard, pheasant, bobwhite quail, and Japanese quail) (131). The experimental results were expressed as LC_{50}s based on 5-day feeding (Table III.6.1). In most cases Aroclor 1260 was significantly more toxic than the less chlorinated mixtures. The bobwhite quail was the most sensitive of the four species tested, but even for this species the LC_{50}s were quite high (Table III.6.1).

Numerous studies have been reported of the toxicity of PCBs to young chickens. Five such studies are summarized in Table III.6.2 (from ref. 129). In a feeding study with 400 ppm Aroclor 1260, only 15% mortality was noted in 60 days (137); in the same conditions Clophen A60 and Phenoclor DP-60 were much more toxic (see next section).

In a study with 10-day old chickens, using Aroclor 1248, mortality after 25 days was 16/30 at 50 ppm in the diet, 4/20 at 40 ppm, 1/30 at

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Table III.6.1 (from ref. 131)

TOXICITY COMPARISONS OF AROCLORS AND FOUR ORGANOCHLORINE PESTICIDES IN DIETS OF 2-WEEK-OLD BIRDS (Heath *et al.*, 1970)

Chemical	LC$_{50}$s^a with 95% confidence limits parenthesized			
	Mallard	Pheasant	Bobwhite Quail	Japanese Quail
Aroclor 1232	—	3150 (2625–3950)	3000 (2575–3500)	>5000
Aroclor 1242	3180 (2610–3880)	2080 (1840–2350)	2100 (1705–2610)	>5000
Aroclor 1248	2795 (2265–3420)	1310 (1165–1480)	1175 (965–1440)	4845 (4355–5410)
Aroclor 1254	2700 (2160–3310)	1090 (965–1230)	605 (410–849)	2900 (2600–3240)
Aroclor 1260	1975 (1265–2750)	1290 (1105–1435)	745 (575–945)	2185 (1915–2480)
Aroclor 1262	3010 (2460–3635)	1235 (1085–1405)	870 (700–1070)	2290 (2040–2575)
DDE	3570 (2810–4670)	840 (730–970)	825 (695–980)	1355 (1110–1650)
DDT	1870 (1500–2370)	310 (255–375)	610 (515–725)	570 (470–690)
Dieldrin	200 (160–240)	55 (49–61)	39 (33–45)	56 (51–61)
Endrin	22 (17–31)	14 (10.5–17.5)	15 (10.5–24)	15 (12.5–17)

[a] LC$_{50}$: ppm chemical in *ad libitum* diet expected to produce 50% mortality in 8 days, 5 days of toxic diet followed by 3 days of untreated diet. Each determination based on 60 birds.

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Table III.6.2 (from ref. 129)

Semichronic Oral Toxicity Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	Other effects	References
Aroclor 42% Cl	Chicken	100, 200, 400, and and 1000 ppm in diet for 4 weeks	0, 0, 50, and 90% respectively	Enlargement; damage at the higher levels	Edema formation from 200 ppm; at high levels internal haemorrhage and tubular dilatation in kidneys	132
Aroclor 42% Cl	Chicken	200 and 400 ppm in diet for 3 weeks	0 and 12% respectively		Pronounced edema at 400 ppm; enlarged kidneys; small spleen; defeathering and dermatitis	133
48% Cl KC-400	Chicken	1, 5, 10, 25. 50, 100. 300, 600, 1200, 2400, and 4800 ppm in diet for 20 days	0% from 1 to 100 ppm; 100% from the 100 ppm level		Edema formation 100 ppm level	134
Aroclor 48% Cl	Chicken	10, 20, 30, 50, 100, and 150 ppm in diet for 4.5–5 weeks	After 3 weeks: 0. 0, 30. 30 and 20%; at the end 0. 0, 80, 60, and 80% respectively	Enlargement	General edema and depression of the secondary sexual characteristics from the 30 ppm level	135
Aroclor 54% Cl	Chicken	250 and 150 ppm in diet for 6 to 13 weeks	250 ppm 100% between 3 and 10 weeks; 500 ppm some mortality at the end		500 ppm at end: comb weights 20-fold and testes weights 2-fold lower than controls	136

30 ppm and 0/10 at 10 ppm. Only 2-4 out of 10 chicks survived on diets containing 100 and 150 ppm (138). In a comparative study with Aroclors 1242, 1254 and 1260, Aroclor 1242 proved the most toxic, causing 100% mortality at 400 ppm in the diet and 40% mortality at 200 ppm in 4 weeks; Aroclor 1254 caused 60% mortality at 400 ppm and 20% mortality at 200 ppm; Aroclor 1260 caused no mortality even at 400 ppm (65). Together these studies show that chickens are more sensitive than any of the species listed in Table III.6.1; also in contrast to these species, chickens are more sensitive to the mixtures with lower chlorination. Marked variations in the toxicity of PCB mixtures to chickens have been noted (2, 136: see next section).

The toxicity of PCBs has been studied in several other species of birds. In Japanese quail, 2000 ppm of Aroclor 1254 in the diet caused 100% mortality between 6 and 55 days (139). In Bengalese finches, a dose rate of about 250 mg/kg/day of Aroclor 1254 was needed to cause 50% mortality in 56 days (140), but the dose-response curve was very shallow and some birds died even at the lowest treatment level (about 6 mg/kg/day). Some mortality was caused among subadult pheasants by doses of Aroclor 1254 as low as 10 mg per day (about 15 mg/kg/day) (141). Cormorants treated with Clophen A60 at 200-400 ppm in the diet died after 55-124 days (142).

Residues of PCBs in the brains of experimentally killed birds were in the range 350-760 ppm in blackbirds (130), 300-400 ppm in pheasants (141) and 75-180 ppm in cormorants (142). Brain residues in these ranges are believed to be indicative of death by PCB poisoning (130). Residues in the livers of poisoned birds were in the range 200-300 ppm in cormorants (142) and 70-700 ppm in Bengalese finches (141), but much higher in pheasants (142).

No investigations appear to have been reported of the toxicity of Aroclor 1016 to birds.

III.6.2 Chick edema disease and the role of PCDFs in PCB toxicity.

The most characteristic signs of toxicity in chickens experimentally poisoned with PCBs are subcutaneous edema, hydropericardium, and in some cases enlargement of the liver, liver necrosis, reduction in the size of the spleen, and kidney damage (Table III.6.2; 65, 129, 132-139). Hydropericardium has also been recorded in some, but not all, treated individuals of Bengalese finches (140), Japanese quail (139) and pheasants (141), but not cormorants (142). Atrophy of the spleen and liver changes were prominent in poisoned pheasants (141). Hemorrhaging in breast muscle and other tissues has also been reported (65, 138).

Edema and hydropericardium are characteristic signs of "chick edema disease", a syndrome which periodically affects flocks of domestic chickens. The cause of "chick edema disease" has been traced to toxic fat included in commercial feeds, and the usual toxic agent has been identified as polychlorinated dibenzodioxins (PCDDs) (143, 67). However, outbreaks of chick edema disease in Japan in 1968 and in the United States in 1971 were traced to contamination of chicken feed with PCBs (144, 65). At least in the latter case, the concentration of PCBs in the feed seemed insufficient to account for its toxicity (65; 2, pp. 327-328). This prompted the search for another toxic contaminant.

Vos and Koeman (137) found that under identical conditions Clophen A60 and Phenoclor DP-6 were much more toxic to chicks than Aroclor 1260, and that most of the classic signs of chick edema disease were caused by the first two mixtures (Table III.6.3). On fractionating the mixtures most of the toxicity was traced to the most polar fractions of Clophen and Phenoclor and compounds tentatively identified as PCDFs (4-Cl and 5-Cl) were identified in them. Subsequently PCDFs have also been identified in the PCBs which caused the Japanese incident in 1968 (34).

TABLE III.6.3 (from ref. 137)

MORTALITY, MEAN SURVIVAL TIME AND PATHOLOGIC OBSERVATIONS OF CHICKS FED 400 ppm PCB FOR 60 DAYS AND OF CONTROL BIRDS

Groups	*N*	Number of deaths	Mean survival[a] time (days)	Number of birds with edema			Number of birds with liver necrosis
				Hydropericardium[b]	Abdominal	Subcutaneous	
I (Phenoclor)	24	24	24.3 (12–58)	18	8	5	9
II (Clophen)	22	22	20.5 (13–29)	20	9	7	9
III (Aroclor)	20	3	—	3	0	0	0
IV (Control)	20	0	—	0	0	0	0

[a] The range of values is shown in parentheses.

[b] Positive hydropericardium response was considered to be over 0.2 ml pericardial fluid in chicks 20 days of age and 0.4 ml in chicks 60 days of age, with interpolation for intermediate ages.

In recent direct studies, 2,3,7,8-tetrachlorodibenzofuran (TCDF) proved exceptionally toxic to chicks (30, 71). Birds which were treated with 5 μg/kg/day TCDF died in an average of 11.5 days. Chicks which were treated with 1 μg/kg/day started to die on the 19th day, and were in very poor condition when the experiment was terminated. The most striking gross pathologic change observed was the accumulation of clear fluid, as evidenced by marked subcutaneous edema, ascites, and hydropericardium, with the severity of the fluid accumulation greatest at the 5 μg/kg dose. The hearts of these birds also appeared enlarged and flabby (rounded). The second striking change was a marked reduction in size of the thymus and spleen. Histologically, size reduction could be accounted for by the marked depletion of lymphocytic cell types in the spleen and cortex of the thymus. Similar pathologic changes were found to occur at the 1 μg/kg dose, albeit to a milder extent. There were no significant effects observed on liver weight; mild liver pathology was found to occur only at the 5 μg/kg dose. Total serum protein levels were reduced with a marked reduction in serum albumin (71).

Although this experiment and that of Vos and Koeman (137) indicate that PCDFs are major causes of chick edema disease, they do not exclude the possibility that some PCBs do so also. For example, Aroclor 1260 caused some deaths and hydropericardium in Vos and Koeman's experiment (Table III.6.3), although the intake of PCDFs by these chicks would have been only about 50 ng/kg/day (400 ppm Aroclor x 0.8 ppm PCDF x 160 g/kg/day food intake; refs. 32, 137). Mortality and hydropericardium have been recorded in other species treated at even lower dose rates of Aroclor 1254 (65, 140, 151). In pheasants, marked atrophy of the spleen, liver enlargement and degeneration, and one case of hydropericardium were induced by daily doses as low as 15 mg/kg/day of Aroclor 1254, an intake which is unlikely to have represented more than 25 ng/kg/day of PCDFs (141, 32).

In another recent study, chicks were dosed with five symmetrical isomers of hexachlorobiphenyl (30, 70). Although four of these had little effect except on the liver (and slight effects on the thymus) at doses of 400 ppm, 3,4,5,3',4',5'-hexachlorobiphenyl proved exceptionally toxic, causing 100% mortality even at 3 ppm in the diet and producing the classic symptoms of chick edema disease at that level (Tables III.6.4, III.6.5). This isomer apparently is not present in commercial PCB mixtures, although structurally similar isomers are present in small quantities (Section II.5.4 above). It is not yet clear whether the effects of this isomer are due to its structural similarity to the most toxic CDFs and CDDs, or whether CDFs may be produced from it by metabolism.

It is therefore impossible with existing information to disentangle the roles of PCBs and PCDFs in chick edema disease. Although PCDFs seem to play a major role in the toxicity of Clophen A60 and Phenoclor DP-6 to chickens (137), the same symptoms are produced by at least one specific PCB isomer. Further, some of the symptoms of chick edema disease are produced by Aroclor mixtures which contain extremely small quantities of PCDFs, and most of the effects on the liver appear to be attributable to PCBs themselves (129). For further discussion of the environmental significance of PCDFs see below in Section III.16.

Chick edema disease has also been induced experimentally with polychlorinated naphthalenes (67) but this effect also is probably attributable at least in part to PCDFs (see Section III.16).

III.6.3. Porphyria and induction of ALA-synthetase

Vos and Koeman (137) found that experimental poisoning of chicks with PCBs was accompanied by increased fecal excretion of coproporphyrin and

TABLE III.6.4 (from ref. 70)

Summary of the biological effects of hexachlorobiphenyl isomers in chicks

Chemical	Dose level (ppm)	Mortality	Body weight gain (% of control)	Liver effect	Porphyrin Accumulation	Thymus effect
3,4,5,3',4',5'-HCB	3	10/10	-	+++	-	+++
	10	10/10	-	++	+++	+++
2,3,4,2',3',4'-HCB	400	0/8	68	++	-	+
2,4,5,2',4',5'-HCB	400	0/10	66	++	-	+
2,3,6,2',3',6'-HCB	400	1/10	80	++	-	+
2,4,6,2',4',6'-HCB	400	0/10	83	+++	-	+

TABLE III.6.5 (from refs. 70, 50)

Summary of major pathological changes in chicks
given different hexachlorobiphenyl isomers for 21 days

Chemical	Dose level (ppm)	Liver	Thymus	Spleen	Fluid Accumulation
3,4,5,3',4',5'-HCB	3	Marked; fatty metamorphosis, focal necrosis.	Marked; involution.	Marked; depletion of lymphocytes.	Subcutaneous edema, ascites, hydropericardium.
2,3,4,2',3',4'-HCB	400	Moderate; hyalinization, single-cell necrosis.	Slight; Involution.	NS[a]	Epicardial edema.
2,4,5,2',4',5'-HCB	400	Moderate; single-cell necrosis.	Slight; involution.	NS[a]	NS[a]
2,3,6,2',3',6'-HCB	400	Moderate; fatty metamorphosis, focal necrosis, giant cell formation.	Slight; involution.	NS[a]	NS[a]
2,4,6,2',4',6'-HCB	400	Marked; fatty metamorphosis, focal necrosis, giant cell formation, marked focal dilatation of sinusoids.	Slight; involution.	NS[a]	NS[a]
2,3,7,8 -TCDF	5 µg/kg	Mild; passive congestion	Marked; involution	Marked; depletion of lymphocytes	Subcutaneous edema, ascites, hydropericardium

[a] NS = not significant.

protoporphyrin and fluorescence of tissues. Chicks which died during the experiment had a much higher incidence of fluorescence in liver, bile, and small intestine than did those that survived; fluorescence in bone was prominent in both groups. The effects were similar in chicks treated with Aroclor 1260, Clophen A60 and Phenoclor DP-6, suggesting that the porphyria is an effect of PCBs themselves rather than PCDFs (129).

In a subsequent study with Japanese quail, Vos et al. (145) found that treatment with Aroclor 1260 at doses of 100 mg/kg/day for 7 days or greater induced porphyrin fluorescence in the liver. Mitochondrial activity of δ-aminolevulinic acid (ALA)-synthetase was greatly increased. Together with the doubling of mitochondrial protein, liver enlargement, and pyroninophilic staining of liver nucleoli, this suggested an increased protein synthesis (145). Further testing at lower doses showed a significant induction of ALA-synthetase at 1 mg/kg and a marked but non-significant increase even at 0.1 mg/kg (Table III.6.6). A significant increase in ALA-synthetase was associated with PCB residues in the liver as low as 1 ppm (Figure III.6.1). This is one of the lowest levels of PCB exposure associated with a measurable effect in an experimental animal.

Testing five individual isomers of hexachlorobiphenyl, Goldstein et al. (146, 147) found that all five stimulated ALA-synthetase, but that there were marked differences in their porphyrinogenic effects (Tables III.6.5, III.6.7). The 3,4,5,3',4',5'- isomer had by far the greatest effect; the 2,4,5,2',4',5'- and 2,3,4,2',3',4'- isomers also had marked effects, but the other two isomers had no significant effects. 2,3,7,8-TCDF had no effect either on ALA-synthetase or porphyrin accumulation (Table III.6.7). The lack of effect of 2,3,7,8-TCDF contrasts strongly with that of the structurally similar 2,3,7,8-TCDD, which is a potent inducer of ALA-synthetase in chicks (148). Furthermore, the

TABLE III.6.6
(from ref. 145)

FORMATION OF δ-AMINOLEVULINIC ACID BY LIVER MITOCHONDRIA, CYTOCHROME P-450, PROTEIN CONTENT OF THE MITOCHONDRIAL FRACTION, LIVER WEIGHTS, LIVER RESIDUES, AND TISSUE FLUORESCENCE IN FEMALE JAPANESE QUAIL ORALLY DOSED WITH PCB FOR 7 DAYS[a]

Aroclor 1260 (mg/kg body weight)	ALA formed (mμmoles ALA/g liver/hr)	Cytochrome P-450 (mμmoles/g liver)	Protein content mitochondrial fraction (mg/g liver)	Liver (g)	Liver (g/100 g body weight)	PCB Content liver (ppm)	Tissue fluorescence incidence	
							Macroscopic	Microscopic (liver)
0	6.46 ± 1.65	<9.1	19.1 ± 5.3	4.43 ± 0.51	3.13 ± 0.13	0.15[c]	0/5	0/5
0.1	8.76 ± 4.06	<6.9	17.7 ± 1.8	4.31 ± 0.62	3.09 ± 0.51	0.54 ± 0.27	0/5	0/5
1	10.50 ± 1.21[b]	<4.3	20.9 ± 3.7	4.22 ± 0.76	2.76 ± 0.35	1.41 ± 0.67	0/5	0/5
10	17.3 ± 6.4[b]	14.0	17.8 ± 2.5	5.09 ± 1.19	3.52 ± 0.65	27.0 ± 9.4	0/5	0/5
100	119.9[c]	19.7[c]	29.1[c]	4.73 ± 0.82	3.89 ± 0.63	478 ± 294	3/5	2/5

[a] Mean values ± SD, 5 birds per group.

[b] Significantly different from controls, $P < 0.01$.

[c] Pooled samples.

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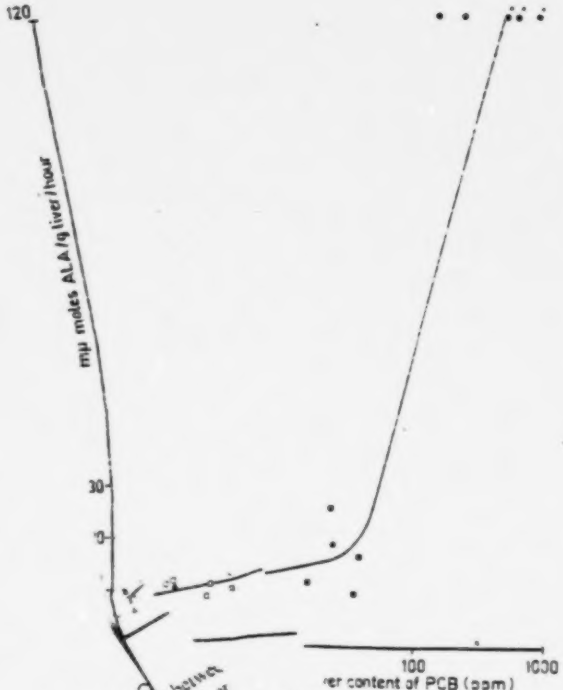
Fig. II]
(from ref.

146



C n betwe
a (mo g liver ent of PCB m) and the mitochondrial ALA synthetase
mg/k = b owing determin nimals: ▲ ng kg: □. 1 mg kg; ■. 10/mg/kg; ●. 100
contrgroup deter ltravio t. Liver samples were pooled in the
tion of he ALA thetase ac ontent, a he 100 mg kg group for the determina-

EFFECT OF 400 PPM PURIFIED HEXACHLOROBIPHENYL ISOMERS AND TCDF ON CHICK LIVERS[a]

Treatment	ALAS nmol/g/hr	Incidence of Porphyria[c] (> 10 μg/g)	Liver Porphyrin Levels (μg/g) (Range)
Control	55 ± 2	0/10	0.4 - 1.4
2,3,6,2',3',6'-HCB	261 ± 15[b]	0/10[d]	0.5 - 1.4
2,4,6,2',4',6'-HCB	303 ± 15[b]	0/10[d]	0.7 - 2.9
2,4,5,2',4',5'-HCB	309 ± 13[b]	3/9 [d]	1.5 - 82
2,3,4,2',3',4'-HCB	250 ± 28[b]	2/8 [d]	1.2 - 81
3,4,5,3',4',5'-HCB	DEAD	1/7 [d]	2.4 - 5,000
Corn Oil	74 ± 2	0/33	0.4 - 1.2
1 μg/kg/day TCDF x 21 days	63 ±20	0/5	0.4 - 2.9
5 μg/kg/day TCDF x 21 days	DEAD	0/5	0.3 - 0.7

[a] Treatment described in Fig. 1.

[b] Significantly greater than controls, $P < 0.05$ (Duncan's Multiple-Range test).

[c] Proportion of animals whose liver porphyrin levels exceed 10μg/g. The upper 99% tolerance levels for controls at $P < 0.01$ is 2.3 μg/g.

[d] Uroporphyrin detectable in all samples after thin-layer chromatography.

Table III.6.7 (from ref. 147)

strong contrast between 3,4,5,3',4',5'-hexachlorobiphenyl and 2,3,7,8-TCDF in their effects on ALA-synthetase and porphyrogenesis (Table III.6.7) show that contamination with the latter compound cannot account for the extreme potency of the former in producing chick edema disease (Table III.6.4).

Only hexa-chlorobiphenyls and Aroclor 1260 appear to have been tested for these effects in birds, but since lower chlorobiphenyls and mixtures are also active in rats (see below) there is no reason to assume that the effects in birds would be limited to the more highly chlorinated compounds.

III.6.4 Effects on reproduction

III.6.4.1 Effects in chickens

Numerous studies have been carried out of the effects of PCBs on reproduction in chickens, because of their apparent sensitivity and because of several commercially damaging incidents (65, 144).

In an experiment reported by Scott *et al*. (149, 150), Aroclor 1248 was fed to White Leghorn chickens at dietary levels of 0.5, 1, 10 and 20 ppm. Egg production was significantly reduced in the 10 and 20 ppm groups after 8 weeks. There was no significant reduction in the breaking strength of eggs at any dose level, but hatchability was severely reduced in the groups fed 10 and 20 ppm. Hatchability was reduced to 72% of normal when the PCB level in the eggs reached 2.2 ppm (wet weight), to about 56% at 3 ppm, and almost to zero at 4.5 ppm.

In another study, White Leghorn and broiler hens were fed diets containing 20 and 50 ppm Aroclor 1242 for six weeks. Even at 20 ppm hatchability was reduced to half that in controls by the second week of feeding and almost to zero in the fourth and fifth weeks. Broiler hens were slightly more resistant than White Leghorns. Most of the embryonic mortality took place in the middle and late stages of development. No hydropericardium was observed in the affected embryos, but abnormal anatomy of the heart was observed, involving ridges on the exterior of the right ventricle (151).*

In another study involving several dose levels of Aroclor 1242, there was no effect on egg production, egg weight, or eggshell thickness at any dose level. Hatchability was reduced in the group fed 10 ppm for six weeks, and reduced markedly in the groups fed 20, 40 or 80 ppm. Significant reductions in hatching success were noted when PCB residues in the yolk exceeded 2.4 ppm (about 1.0 ppm in the whole egg). Hatchability recovered almost to normal after a 6-week period on clean food in which the yolk residues fell below 1.8 ppm (152).

In another study involving feeding Aroclor 1254 at dietary levels of 5 and 50 ppm, egg production and fertility were reduced at both exposure levels; hatchability of fertile eggs was affected only in the 50 ppm group (153).

In another study in which Aroclor 1254 was included in drinking water at 50 ppm (corresponding to an intake of about 6.3 mg/kg body wt/day), fertility was unaffected but hatchability dropped to zero by the third week. Hatchability started to increase after nine weeks on uncontaminated diets, but did not return to normal until about the 17th week, when yolk residues had declined to about 10 ppm (Figure III.6.2). Deformities of the legs, toes, and neck were observed in many chicks hatching from eggs with yolk levels 10-15 ppm or more (154, 155). Other developmental defects were observed in the few chicks surviving these concentrations (154).** Teratogenic effects were also observed when 5 mg Aroclor 1242 was injected into the yolk sac of fertile eggs (156).

In a comparative study, White Leghorn chickens were treated with Aroclors 1016, 1232, 1242, 1248 and 1254 for eight weeks at dietary levels

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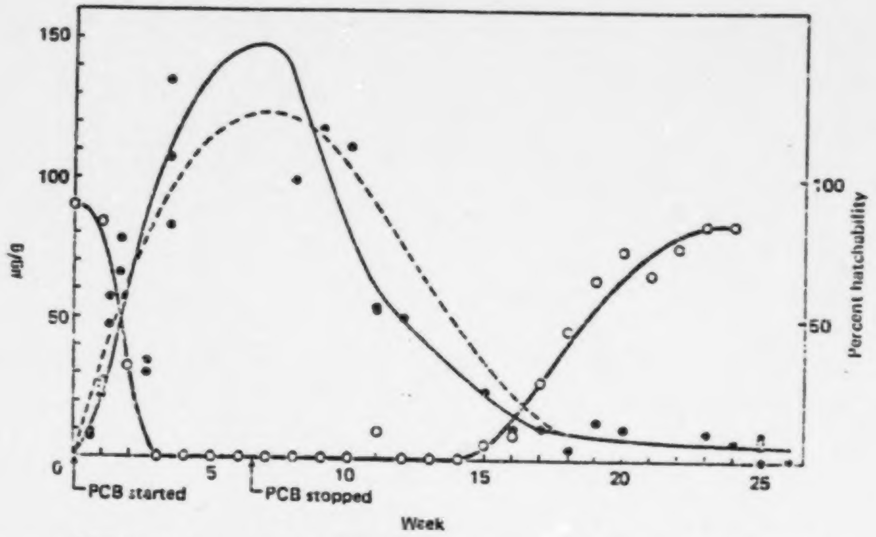
* Abnormalities of the epicardium (fenestration and fibrous degeneration) were reported in another study involving feeding of Aroclor 1254 (400).

** Toe deformities were also seen in birds of the second (F_2) generation, suggesting a mutagenic effect (155, p. 204).

Figure III.6.2
(from ref. 155)



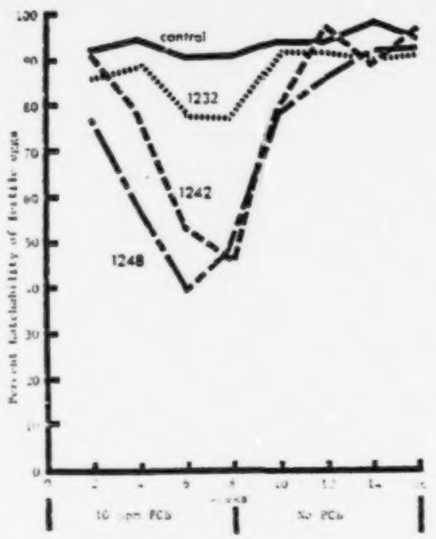
PCB levels in egg yolk, determined by TLC, and hatchability of experimental eggs. ● = PCB level; ———— = hand-interpolated curve; – – – – = least-squares fit to a third-order regression; ○ = hatchability.

of 5, 10 and 20 ppm. None of the mixtures affected egg production, egg weight, eggshell thickness or food consumption. Hatchability of fertile eggs was severely reduced by Aroclors 1232, 1242 and 1248 at the 10 or 20 ppm levels (Fig. III.6.3), but there were no significant effects induced at 5 ppm, nor by Aroclors 1016 or 1254 at any level. The growth rate of the surviving chicks was reduced by maternal exposure to all 5 mixtures (chicks were exposed only via the egg); this reduction was most marked for Aroclors 1242 and 1248. Embryonic death was most pronounced in two periods of development: on days 3-10 and after the eggs were pipped (Table III.6.8). Abnormal embryos were found in eggs treated with Aroclors 1242 and 1248 (Table III.6.8): abnormalities included edema, unabsorbed yolk, small bodies, external hemorrhage, and leg malformations (157). In an earlier study with 20 ppm Aroclors 1242 and 1248 as many as 33-50% of the dead embryos showed these abnormalities (158). In this earlier study Aroclors 1221 and 1268 appeared to have no effects on chicken reproduction at levels of 20 ppm (158).

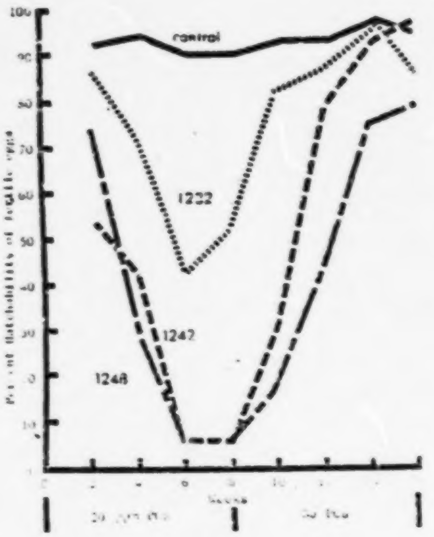
In another comparative study with White Leghorn chickens no effects were found with Aroclor 1260 at any dose level up to 100 ppm; Aroclor 1254 affected egg production and hatchability at 100 ppm, and chick viability was reduced at 10 ppm; Aroclor 1242 reduced egg production at 100 ppm, chick viability at 10 ppm, and hatchability at 8 ppm, but not at 4 ppm (68). Eggshell thickness is said to have been reduced by Aroclor 1242 at 10 and 100 ppm, but no details were given and this result conflicts with that obtained by other experimenters.

Finally, in the East Coast Terminal incident in which commercial chicken feed was contaminated with Aroclor 1248 (65), hatchability of eggs from chickens ingesting the feed dropped to about 2% (2). In affected

Figure III.6.3
(from ref. 157)



Effect of 10 p.p.m. PCB feeding and subsequent PCB withdrawal on hatchability of fertile eggs in a 16-week period.



Effect of 20 p.p.m. PCB feeding and subsequent PCB withdrawal on hatchability of fertile eggs in a 16-week period.

TABLE III.6.8
(from ref. 157)

—Fertility, hatchability, abnormal embryos and peak of embryonic death during feeding of Aroclors 1242 and 1248 in the maternal diet

PCB	PCB level	Fertile eggs	Hatch	Abnormal embryos as % of		Embryonic death % on incubation day				Pipped eggs
Aroclor	p.p.m.	Number	% of fertile eggs	Fertile eggs	Dead embryos	1-2	3-10	11-19	20-21	% of day 21 dead
None	0	163	91	2	20	53	7	26	14	0
1242	5	106	76	8	35	54	15	0	31	33
1248	5	109	87	2	14	36	21	7	36	40
1242	10	103	67	9	26	12	47	9	32	43
1248	10	114	69	5	17	11	40	0	49	29
1242	20	194	28	12	17	4	38	13	45	29
1248	20	204	22	13	16	9	38	9	45	31

samples of eggs, fresh yolk prior to incubation contained about 0.29 ppm, yolk of fertile eggs contained 0.97 ppm and 1-day-old dead embryos contained 0.35 ppm (2). Comparison with the levels required to reduce hatchability in other experiments (149, 150) indicates that the material which leaked from the heat exchanger was considerably more toxic than fresh Aroclor 1248 (2: see Section II.8.4 above).

To summarize these studies with chickens, marked effects on reproductive success have been noted at dietary levels as low as 8-10 ppm, corresponding to residues of only 1-2 ppm in whole eggs. Aroclors 1242 and 1248 are much more potent than higher or lower chlorinated mixtures, but Aroclor 1016 was much less active than Aroclor 1242 in one test. The principal cause of reproductive failure is embryonic mortality, and abnormal embryos and teratogenic effects have been reported in several experiments.

The possible role of PCDFs in embryonic mortality is not clear. PCDFs appear to be embryotoxic in chickens (129) and the high toxicity of the PCBs in the East Coast Terminal incident suggests the presence of a toxic contaminant (2). However, it is difficult to attribute the greater toxicity of Aroclor 1242 than Aroclor 1016 (157) solely to the presence of PCDFs in the former mixture, because Aroclors 1254 and 1260 (which also contain PCDFs - 32) appear much less embryotoxic (157, 68). It seems more plausible to attribute the high toxicity of Aroclors 1242 and 1248 in chicken embryos to the presence of a specific tetrachlorobiphenyl which is less well represented in Aroclors 1016 and 1254. Ax and Hansen (399) report that 2,4,5,3',4'- pentaCB is very toxic to chick embryos, but this isomer is not present in Aroclor 1242.

III.6.4.2 Effects in Pheasants

Dahlgren _et al_ (141, 159) dosed female pheasants with Aroclor 1254 by capsule at rates of 12.5 or 50 mg weekly (approximately 2.5 and 10 mg/kg/day, equivalent to about 50 and 200 ppm in the diet). Egg production was significantly reduced in all treated groups, and there was a significant increase in the number of eggs that were pipped but not hatched. Although egg fertility and hatchability were not significantly reduced, survival of chicks was reduced in all groups and the overall reproductive success was significantly reduced. No effects were found on eggshell thickness (141).

III.6.4.3 Effects on Mallards

In a two-year study with Mallards, Aroclor 1254 at 25 ppm in the diet had no significant effects on any aspect of reproductive success except for a reduction in the number of eggs laid, which took place only in one of four pens and was believed to be unrelated to treatment (131) (Table III. 6.9). Eggshell thickness was unaffected by Aroclor 1254, although in a parallel experiment DDE caused significant eggshell thinning (Table III.6.10). Residue levels in eggs (33 ppm wet weight) were much higher than those associated with embryonic mortality in chickens.

Similar results were reported in another experiment in which Mallards were fed 40 ppm Aroclor 1254, 40 ppm DDE, and the two compounds together (160). Unlike DDE, Aroclor 1254 did not cause significant eggshell thinning, and it caused only a slight and non-significant increase in the degree of eggshell-thinning induced by DDE (Table III.6.11). However, egg production was markedly reduced in the Mallards fed PCBs and DDE simultaneously, and there was a significant increase in egg-eating by the birds on combined dosage (160).

III.6.4.4. Effects in Bobwhite Quail

Reproduction in bobwhite quail was not measurably affected by dietary exposure to either Aroclor 1254 or DDE, administered singly or in combination (131) (Table III.6.12).

TABLE III.6.9
(ref. 131)

REPRODUCTIVE SUCCESS AMONG PENNED MALLARDS MAINTAINED FOR TWO SEASONS[1] ON FEEDS CONTAINING AROCLOR 1254 OR DDE

	season	chemical added to feed (ppm) control	Aroclor 1254 (25)	DDE (10)
Pens of birds	1969	4	4	4
(5 ♀♀, 2 ♂♂ each)	1970	4	4	4
Hen-seasons	1969	20	20	19.1
(8 weeks each)	1970	20	19	18
Total eggs laid	1969	590	433	623
	1970	630	468	632
Eggs laid per	1969	29.5	21.7	32.8
hen-seasons	1970	31.5	24.6	35.1
Eggs cracked (%)	1969	4	6	20[2]
	1970	9	9	28[2]
Eggs embryonated of	1969	86	94	80
eggs set (%)	1970	88	90	68
Embryos alive at	1969	98	96	94
3 weeks (%)	1970	92	92	88
Normal hatchlings of	1969	70	63	47[2]
3-week embryos (%)	1970	59	64	53
Normal hatchlings alive	1969	94	91	97
at 14 days (%)	1970	93	96	92
14-day ducklings of	1969	64	55	42[2]
embryonated eggs (%)	1970	5[illegible]	57	43[2]
14-day ducklings per	1969	13.0	8.8	7.8[2]
hen-seasons	1970	11.6	10.4	6.7[2]

[1] Dosage started December 19, 1968; eggs collected from March 12 through May 6, 1969, and from March 18 through May 12, 1970

[2] Differences from controls statistically significant ($P < 0.05$).

TABLE III.6.10
(ref. 131)

MEAN THICKNESS OF EGGSHELLS OF PENNED MALLARDS MAINTAINED THROUGH TWO SEASONS ON FEED CONTAINING AROCLOR 1254 OR DDE

		chemical added to feed (ppm)		
	season	none	Aroclor (25)	DDE (10)
No. of eggs	1969	28	27	27
	1970	32	17	26
Shell thickness (mm)	1969	0.375	0.360	0.339[1]
	1970	0.365	0.368	0.328[1]

[1] Differences from controls statistically significant ($P < 0.05$).

TABLE III.6.11
(from ref. 160)

Mean eggshell thickness indices for 1-day egg collections from cages of mallards fed four experimental diets.

Treatment	Single 1-day egg collections						Largest 1-day collections during 3-day periods[a]			
	Day 30		Day 32		Day 50		Days 30–32		Days 50–52	
	No. eggs	Mean index[b]	No. eggs	Mean index	No. eggs	Mean index	No. eggs	Mean index	No. eggs	Mean index
Control	7	0.212 A	5	0.214 A	5	0.213 A	9	0.212 A	8	0.217 A
PCB	4	0.193 AB	4	0.211 AB	8	0.216 AB	5	0.201 AB	9	0.216 AB
DDE	6	0.177 BC	5	0.150 C	7	0.170 C	6	0.177 C	8	0.172 C
DDE + PCB	8	0.164 C	6	0.170 C	8	0.166 C	9	0.167 C	8	0.166 C
F-values[c]		7.73		12.00		26.00		18.50		22.00

[a] Values for each treatment represent combined data for the single largest 1-day egg collection during the 3-day period from each of two replicate cages.

[b] Within a column, means followed by the same letter are not significantly different ($P < 0.05$).

[c] All F-values are significant ($P < 0.005$).

III.6.4.5 Effects in Ring Doves

Peakall et al. (161) studied reproduction in ring doves exposed to 10 ppm Aroclor 1254 for two generations. Although there were no effects in the first generation, there was high embryonic mortality in the second generation (Table III.6.13). Cytogenetic studies of treated embryos showed a number of chromosomal aberrations (primarily chromatid, some isochromatid, and one rearrangement). Thirteen of 17 treated embryos had aberration rates exceeding the mean rate in controls (161). Eggshell thickness was not affected (162). In a subsequent study it was found that embryonic mortality was due in part to abnormal incubation behavior by the PCB-treated parents (163). Eggs incubated artificially, or by untreated parents, had a higher hatching success. However, significant embryonic mortality was observed even in artificially incubated eggs, and this was attributed primarily to the chromosomal effects (163).

III.6.4.6 Effects in American Kestrels

Because residue levels of PCBs are often very high in predatory birds (1, 2, 6) special interest attaches to an experiment in which the effects of dietary PCBs were studied in captive American Kestrels (164). Groups of breeding Kestrels were fed diets containing 10 ppm Aroclor 1254 (wet weight of diet), 3 ppm DDE, and a combination of the two chemicals. Eggshell-thickness was slightly increased in the PCB-treated birds (statistically significant in one experiment, but not in others), markedly decreased in the DDE-treated birds, and still further decreased in the birds treated with DDE and PCBs (Table III.6.14). The difference between the birds treated with DDE and those treated with both chemicals was statistically significant. All the eggs from birds treated with both chemicals were broken during incubation (Table III.6.14). When eggs were artifically incubated, embryonic

TABLE III.6.12
(from ref. 131)

REPRODUCTIVE SUCCESS AMONG PENNED BOBWHITE FED DIETS CONTAINING AROCLOR 1254, DDE, OR A MIXTURE OF BOTH CHEMICALS[1]

		chemical added to feed (ppm)		
	control	Aroclor (50)	DDE (50)	Aroclor 1254 + DDE (25) + (15)
Pens of birds (3 ♀♀, 2 ♂♂ each)	6	6	6	5
Hen-seasons (8 weeks each)	16.6	16.4	15.3	14.9
Total eggs laid	347	368	415	386
Eggs laid per hen-season	20.9	22.4	27.1	26.0
Eggs cracked (%)	10	7	5	7
Eggs embryonated of eggs set (%)	88	90	93	93
Embryos alive at 3 weeks (%)	97	97	98	97
Normal hatchlings of 3-week embryos (%)	96	91	96	92
Normal hatchlings alive after 14 days (%)	78	72	70	71
14-day chicks of embryonated eggs (%)	73	64	66	65
14-day chicks per hen-season	21.5	11.5	15.1	13.5

[1] Dosage started April 3, 1968; eggs collected from April 27 through June 21, 1968.

None of the above differences was significant at the 95 percent level of confidence.

MEAN THICKNESS OF EGGSHELLS OF PENNED BOBWHITE MAINTAINED THROUGH ONE SEASON ON FEED CONTAINING AROCLOR 1254, DDE, OR A MIXTURE OF BOTH CHEMICALS

		chemical added to feed (ppm)		
	none	Aroclor (50)	DDE (30)	Aroclor 1254 + DDE (25) + (15)
No. of eggs	14	17	20	18
Shell thickness (mm)	0.207	0.208	0.205	0.215

None of the above differences was significant at the 95 percent level of confidence.

TABLE III.6.13
(from ref. 172)

Breeding success of Ring Doves fed 10 ppm PCBs over two generations.

	No. of pairs	No. of eggs laid	No. of eggs hatched	No. of young fledged
Pre-experiment	6	24	22	22
1st generation PCBs	6	24	24	24
2nd generation PCBs	6	20*	4	2**
2nd generation Control	6	24	22	22

* One pair failed to produce any eggs during the period of the experiment.

** Fledging losses consisted of one young found outside the nest at one day of age and one deformed bird killed at the age of three weeks.

Table III.6.14 (from ref. 164)

Kestrel Eggshell Thinning in Response to Chronic Dietary p,p'-DDE and/or Aroclor 1254 Based on (A) Ratcliffe's Index*, (B) Direct Measurement of Shell With Membranes$^{(mm)}$, (C) Incidence of Egg Breakage**.

(A)

	Control	3 ppm DDE	10 ppm 1254	3 ppm DDE + 10 ppm 1254
# of Eggs	15	17	18	12
$\bar{x} \pm$ s.d.	0.920 ± .050	0.799 ± .054	0.977 ± .080	0.770 ± .045
Standard Error	0.010	0.010	0.017	0.010
% Difference from Control	0.00	-13.16	+6.19	-16.31
Statistical Significance^{tt}	—	< .0005	< .005	< .0005

(B)

# of Eggs	15	18	14	15
$\bar{x} \pm$	0.171 ± .013	0.145 ± .009	0.176 ± .015	0.139 ± .008
Standard Error	0.003	0.002	0.004	0.002
% Difference from Control	0.00	-15.21	+2.92	-18.72
Statistical Significance^{tt}	—	< .0005	< .025	< .0005

(C)

	0(3)	0(5)	0^{t}(5)	100(5)

* Oven dry shell weight (mg/length x breadth of egg (mm).

** Percent of total number of pairs; number of pairs in parentheses.

t One pair exhibited breakage but was attributable to other causes.

tt Probability of a larger Student's t value when compared with controls using a one-tailed t-test (Steel and Torrie, 1960; Table A. 3).

mortality was increased in all treated groups but was greatest (100%) in the eggs from birds treated with both chemicals (164). Thus, while 10 ppm Aroclor 1254 appeared to cause some embryonic mortality, it also significantly potentiated the effects of DDE in causing eggshell thinning, egg breakage, and embryonic mortality. PCB residues in the eggs of the jointly-dosed groups averaged 184 ppm (dry weight), i.e., about 30 ppm, wet weight.

III.6.4.7 Effects in Japanese quail

Japanese quail appear relatively resistant to reproductive effects of PCBs and have been tested only at high dietary levels. Dietary exposure to 312 ppm Aroclor 1242, 78 ppm Aroclor 1254, and 62.5 ppm Aroclor 1260 caused a significant reduction in egg production and significant reductions in eggshell thickness (by 4%, 12% and 10% respectively). Egg weights were also reduced in birds fed Aroclor 1260 (165). In another experiment 100 ppm Aroclor 1242 caused a reduction in egg production, but 100 ppm Aroclor 1254 did not. Aroclor 1242 increased the number of broken eggs (166). Dietary exposure to 50 ppm Aroclor 1254 caused only a slight and non-significant reduction (4.5%) in eggshell thickness (167).

III.6.4.8 Summary of effects on reproduction in birds

There are marked differences between species in sensitivity to PCBs. Chickens are very sensitive, particularly to Aroclors 1242 and 1248; their reproduction is markedly affected by dietary levels of 8-10 ppm, corresponding to residues of 1-4 ppm in eggs. Ring doves were seriously affected by dietary exposure to 10 ppm over two generations, and American Kestrels by exposure to 10 ppm in combination with DDE. In contrast, mallards, bobwhite quail and Japanese quail are much less affected by PCBs. There is little evidence of significant effects on eggshell thickness, except in kestrels in combination with DDE. Indications of synergistic effects between PCBs and DDE on reproduction in kestrels and mallards may have important ecological implications, as may the second-generation effects in ring doves.

III.6.5 Liver and microsomal enzymes

The liver has been the primary organ used in delineating effects of PCBs on birds. This concentration of interest can be attributed to the high liver PCB levels found, and to the responsibility of the hepatic systems for metabolizing foreign compounds. Increases in liver weight and size are commonly noted (157, 168). Increase in amount of liver lipids present and a decrease in the storage of liver vitamin A have been frequently reported (158, 166). The liver vitamin A storage reduction has not evidenced symptoms of vitamin A deficiency. However, it was speculated that birds intaking marginal dietary levels of vitamin A, coupled with the liver vitamin A storage reduction, might be susceptible to avitaminosis.

Elevations of the liver cytoplasmic RNA content in PCB-treated birds have been related and correlated with increases in hepatic enzyme activity. Lower cytophotometric readings of percent transmission in experimental livers was determined to be a consequence of increased cytoplasmic RNA (169). Bailey and Bunyan (170) noted increased cytochromeP-450 levels which are indicative of microsomal induction in the livers of birds given PCBs. These elevated levels existed for six months after the withdrawal of dietary PCBs indicating that extremely low PCB residues can cause significant increases in microsomal enzymatic activity.

Several hepatic enzymes have been reported to be induced by PCBs. Evaluation of the liver enzyme controlling pentobarbital metabolism (166) revealed that PCBs exhibit a diphasic effect on pentobarbital induced sleeping times. The PCBs initially inhibit the enzymes metabolizing pentobarbital. Subsequently, these same enzymes are induced, by the PCB stimulation, to greater activity yielding more rapid than normal metabolism of pentobarbital.

Estradiol degradation is another function which PCBs have been found to alter. Lincer and Peakall (169) reported a dose-dependent breakdown of estradiol to a more polar metabolite. Nowicki and Norman (171) reported increased *in vitro* hepatic metabolism of two additional natural steroids: testosterone and 4-androstene-3,17-dione. These two were reduced to polar metabolites as was estradiol. The biological effects of this increase in metabolism of sex hormones to polar forms is relatively unknown but it may be a significant contribution to reproductive problems and failures that have been reported.

A study of hepatically-directed plasma enzyme activity was conducted by Dieter (172). Sublethal doses in quail produced elevation in enzyme activity and reductions in hematocrit and hemoglobin concentrations. The plasma enzymes increased were: (1) cholinesterase by 80%; (2) fructosediphosphate aldolase by 5 to 6 fold; (3) creatine kinase, 4 fold; (4) lactate dehydrogenase, 9 fold; and (5) aspartate aminotransferase by 75%. ChE, LDH, and AAT elevations were dose-related enzyme releases which could be used to quantitatively establish PCB exposure. Qualitative exposure assays were judged possible due to differentiation of enzyme activity in birds exposed to DDE, malathion, mercuric chloride and Aroclor 1254.

Goldstein *et al*. (147) studied the effects of five isomeric hexachlorobiphenyls and 2,3,7,8-TCDF on liver enzymes in the chicken in order to elucidate structure-activity relationships. All the hexa-CB isomers induced a number of parameters of drug metabolism in chicks including cytochrome P-450, increased liver weight, and p-nitrophenyl glucuronyl transferase, but not testosterone glucuronyl transferase activity. The most active inducers were the 2,3,4,2',3',4'- and 2,4,6,2',4',6'- isomers (Figures III.6.4 and III.6.5). The effects of 2,3,7,8-TCDF were both quantitatively and qualitatively different: it induced only a small increase in cytochrome P-450 and this cytochrome differed from that induced by the hexa-CBs, differing by a shift in the peak of the CO-difference spectrum from 450 nm to 449 nm and in characteristics of the ethyl isocyanide difference spectra (Table III.6.15). This is again important in showing that PCBs and PCDFs have independent toxic effects.

III.6.6 Other toxic effects in birds

Alteration of the thyroid has been reported in conjunction with PCB exposure. Hurst et al. (168) found depression of thyroid size, with low dosages or early in the experiment, followed by stimulation of thyroid growth yielding increased size with higher dose levels or later in the study. Increased thyroid weight and size in treated gulls were also reported by Jefferies and Parslow (173). These elevations did not appear to be dose-related and were accompanied by large, colloid-filled thyroid follicles. This condition parallels simple goiter resulting from insufficient intake or utilization of iodine. Decreased mean heart weights were tabulated which would support the possibility of hypothyroidism suggested by the involution of the thyroid tissues and by a minimal decrease in secretion. Jefferies (174) has recently reviewed the role of the thyroid in the production of sublethal effects by PCBs and has suggested that effects on the thyroid may be linked with several other effects, including changes in vitamin A storage, decreased reproductive success and effects on behavior.

Cardiovascular effects of PCBs on birds were evaluated by Iturri _et al_. (175). Heart rate was reduced by Aroclors 1242 and 1254, but not affected by Aroclors 1221 or 1260. Aroclor 1254 lowered arterial blood pH significantly, which can lower cholinesterase activity.

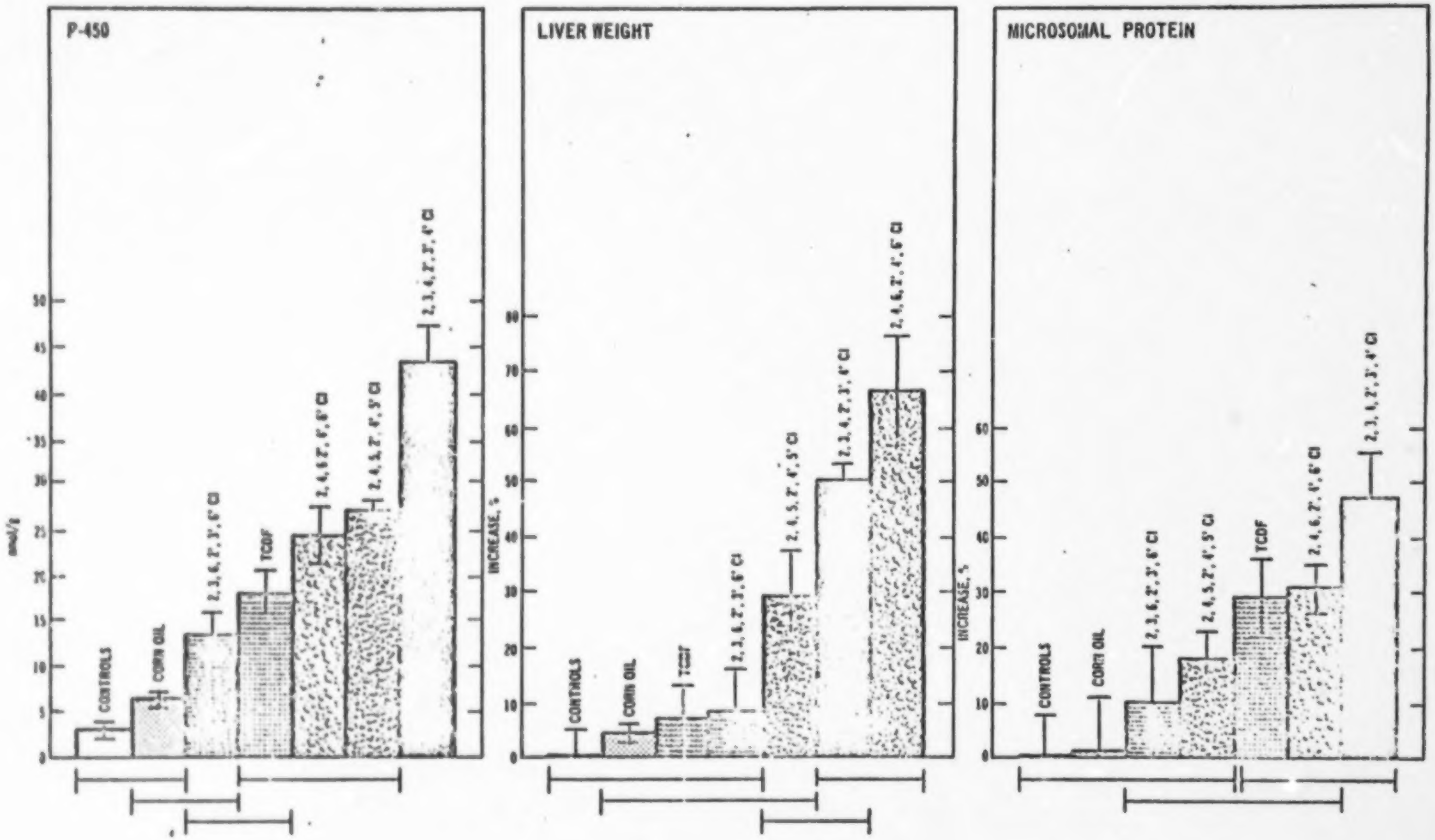


Figure III.6.4. Effects of purified hexachlorobiphenyl isomers and TCDF on chick livers (147). The heavy lines below the bar graph indicate groups which do not differ significantly from each other.

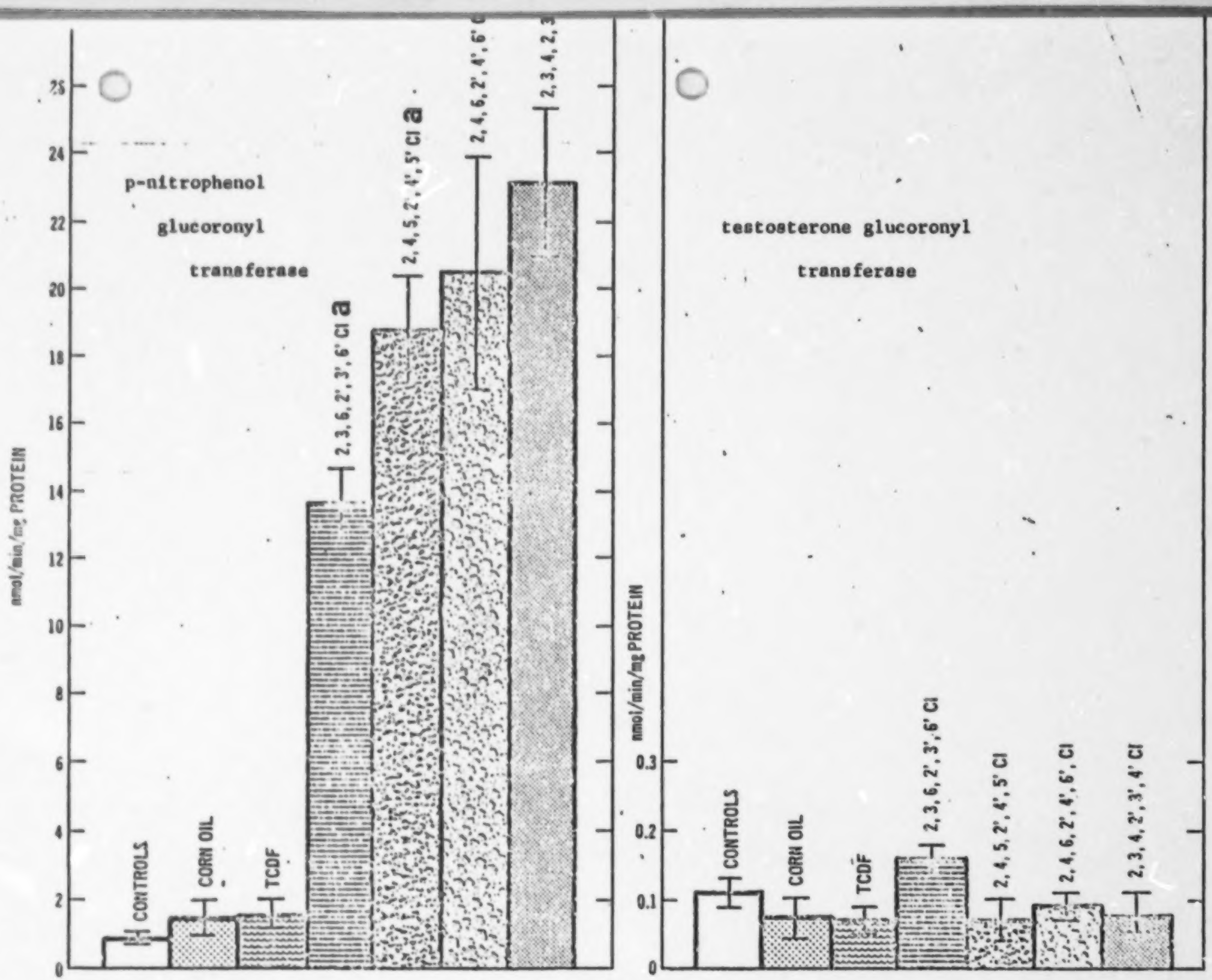


Figure III.6.5 (from ref. 147) Effects of hexachlorobiphenyl isomers and TCDF on chick liver enzymes.

Table III.6.15 (from ref. 147)

EFFECTS OF HEXACHLOROBIPHENYL ISOMERS AND TCDF ON ETHYL ISOCYANIDE DIFFERENCE SPECTRA IN THE CHICK[a]

TREATMENT	455/430 RATIO
Controls	0.42 ± 0.01
2,4,5,2',4',5'-HCB	0.40 ± 0.04
2,3,6,2',3',6'-HCB	0.40 ± 0.10
2,4,6,2',4',6',-HCB	0.37 ± 0.01
2,3,4,2',3',4'-HCB	0.43 ± 0.01
Corn Oil	0.51 ± 0.07
1 μg/kg TCDF	0.74 ± 0.06[b]

[a] Treatment is described in Figure 1. Values represent the means ± SE.

[b] Significantly different from controls, $P < 0.05$ (Duncan's Multiple-Range test).

everal authors have reported sublethal effects of PCBs on behavior. exposure of ring doves to 10 ppm Aroclor 1254 disrupted their in- ion behavior, which caused intermittent chilling of eggs and contributed ificantly to hatching failure (163). A similar phenomenon appeared to significant in American kestrels (164) Low doses of Clophen A50 (0.25 g/kg/day in the diet) are reported to have increased the nocturnal activity of migrating robins (176). Pheasant chicks hatched from hens fed 50 mg of Aroclor 1254 weekly made significantly more inappropriate choices in a visual cliff test than did controls, and were significantly easier to catch by hand in their pens (141, 159)*. Exposure to Aroclor 1254 also significantly impaired the escape responses of quail chicks when presented with strange objects (73). Although these behavioral effects may have little effect on the performance of birds in aviary situations, they have potentially large ecological significance for wild birds under natural environmental stresses.

Harris _et al_. (177) found a reduction in the size of the spleen and bursa of Fabricius in chicks hatched from chickens fed 10 and 20 ppm of Aroclors 1232, 1242, 1248, 1254 (Table III.6.16). As in earlier experiments (157) the effects of Aroclor 1016 were smaller than those of Aroclor 1242 and not statistically significant. An effect on the bursa of Fabricius was noted even at 5 ppm Aroclor 1248 (Table III.6.16) -- a level corresponding to a residue of only about 1.5 ppm of PCBs in the eggs (150). The bursa is the lymphoid organ responsible for establishing immunoglobin synthesis, but Harris _et al_. did not find a clear effect of PCB treatment on antibody production (177). However, Friend and Trainer (178) found that PCB-treatment increased the susceptibility of ducks to viral hepatitis.

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* The effect on escape behavior was also marked when the male parent was treated, suggesting a possible mutagenic effect (141).

Table III.6.16 (from ref. 177)

Hatchability of eggs and bursa of Fabricius and spleen weights of chicks from hens fed Aroclors for 8 weeks (Experiment 1).

treatment roclor[a])	PCB level (p.p.m.)	Hatchability of eggs (percent)	n	Progeny organ weights bursa weight mg±SE	 spleen weight mg±SE
None	0	90	15	62.7 ± 4.0	17.0 ± 1.1
1232	5	90	5	54.1 ± 4.0	17.0 ± 0.6
	10	77[b]	5	59.2 ± 1.5	12.4 ± 0.8[b]
	20	51[b]	10	43.7 ± 3.5[b]	13.8 ± 0.8[b]
1016	5	83	5	49.0 ± 4.8	15.1 ± 1.1
	10	87	5	46.5 ± 3.2	13.8 ± 1.3
	20	92	5	77.8 ± 2.8	20.0 ± 0.7
1242	5	87	5	59.0 ± 9.6	16.4 ± 1.0
	10	46[b]	10	40.3 ± 3.2[b]	13.6 ± 0.9[b]
	20	4[b]	8	36.3 ± 2.5[b]	14.5 ± 1.2
1248	5	86	5	39.1 ± 3.3[b]	16.7 ± 2.3
	10	48[b]	10	42.5 ± 3.8[b]	13.5 ± 1.0[b]
	20	6[b]	5	36.3 ± 4.6[b]	13.6 ± 2.2[b]
1254	5	98	5	52.8 ± 1.6	15.3 ± 1.4
	10	88	5	48.7 ± 4.9	18.1 ± 1.4
	20	88	10	41.2 ± 2.9[b]	14.5 ± 0.7[b]

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[a] Last two digits of Aroclor designate the % Cl, except Aroclor 1016 which contains 41% Cl.

[b] Significantly different ($p<0.05$) from the unsupplemented control using Student's "t" test.

Combs et al. (397, 398) found that 10 ppm of Aroclor 1254 in the diets of female chickens increased the susceptibility of their offspring to vitamin E-selenium deficiency when the chicks were reared on a diet deficient in vitamin E and supplemented with a marginal level of selenium. Susceptibility to this deficiency, as measured by the incidence of exudative diathesis, was also increased when PCBs were added to chick diets. PCBs at 10 ppm in the diet caused a tenfold increase in benzopyrene hydroxylase activity and this led to a decrease in the biological utilization of dietary selenium. The mechanism of the effect appeared to be oxidation of selenium, leading to reduction in its incorporation into glutathione peroxidase and its conversion into selenium compounds of limited biological usefulness (398).

III.7. Toxic Effects in Mammals: Acute and Subacute Studies

III.7.1. Acute toxicity

PCBs do not have high acute toxicity. Table III.7.1 summarizes measurements of the single-dose oral LD_{50} in rats and the single-dose dermal MLD in rabbits, for a range of Aroclor mixtures, as reported by the U.S. Food and Drug Administration (1). Weanling rats are more sensitive: the single-dose oral LD_{50}s for Aroclors 1254 and 1260 are 1,295 and 1,315 mg/kg (179). The single-dose i.v. LD_{50} for adult rats was 358 mg/kg (179). Grant and Phillips (180) obtained similar values for oral LD_{50} of Aroclor 1254 in 30-day-old rats (1,300-1,400 mg/kg) and higher values for 120-day-old rats (2,000-2,500 mg/kg).

The single-dose oral LD_{50} for Kanechlor KC-400 in mice was reported to be about 2,000 mg/kg (181).

III.7.2. Subacute toxicity

Table III.7.2 summarizes reports of the subacute toxicity of PCBs available in 1972 (129, summarizing data from refs. 181-186). Together these data show that PCBs are toxic to mammals by ingestion, dermal absorption, and inhalation. Guinea pigs seem to be much more susceptible than rats and mice. Among signs of toxicity, liver damage is the most consistently observed (Table III.7.2). However, more thorough examination of rats killed by single doses of 4-10 g/kg of Aroclors 1254 and 1260 revealed hemorrhage into the lung, stomach and pancreas (187). Foci of ulceration surrounded by a severe inflammatory reaction were observed primarily in the duodenum and occasionally in the glandular part of the stomach.

TOXICITY OF AROCLORS*.

	Aroclors										
	1221	1232	1242	1248	1260	1262	1268	4465	5442	5460	2565
Oral LD_{50} mg/Kg (rats)	3980[a]	4470[a]	8650[a]	11,000[a]	10,000[b]	11,300[b]	10,900[b]	16,000[b]	10,600[b]	19,200[c]	6,310[c]
Skin MLD mg/Kg (rabbits)	>2000[a]	>1260[a]	>794[a]	>794[a]	>1260[b]	>1260[b]		>2000[b]	>1260[b]	>7940[c]	>2000[c]
	<3169[a]	<2000[a]	<1269[a]	>1269[a]	<2000[b]	<3160[b]	>2500[c]	<3160[b]	<2000[b]		<3160

[a] Undiluted.

[b] Administered as 50% solution in corn oil.

[c] Administered as 33.3% solution in corn oil.

*FDA Status Report on the Chemistry and Toxicology of Polychlorinated Biphenyls (PCB) or Aroclors as of June 1, 1970.

Table III.7.1 (ref. 2)

Table III.7.2 (ref. 129)

Reproduced from best available copy.

Acute and Subacute Oral Toxicity Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	References
Unknown	Mouse	single dose of approx. 2000 mg/kg	LD50		181
Aroclor 54% Cl	Rat	single dose of 500 mg/kg	0%	Increase of weight and lipid; potentiation of CCl_4 toxicity	182
Aroclor 42, 54, 60, and 68% Cl	Mallard	single dose of 2000 mg/kg	0%		183
42% Cl	Rat	20 daily doses of 138 mg	0% in 3 months	Hyalin bodies in liver cells	184
42% Cl	Guinea pig	2 doses of 60 mg 1 week apart	100% between 11 and 29 days	Fatty metamorphosis; central atrophy	184
65% Cl	Rat	6 daily doses of 300 mg	70% in 14 days	Increase of weight; cell swelling; hyalin granules	185
65% Cl	Rat	Doses of 50 mg every second day	60% in 5 weeks	23% weight increase; cell swelling; hyalin globules	185

Dermal Toxicity and Inhalation Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	Skin effects	References
42% Cl	Guinea pig	11 daily skin applications of 34.5 mg	100% between 11 and 21 days	Fat; central atrophy; perinuclear basophilic granulation; focal necrosis in a few animals	Occasional thickening of the epidermis	184
42% Cl	Rabbit	Skin application at alternate days, total dose from 946 to 1980 mg	100% between 17 and 98 days	Fatty degeneration; central atrophy	Thinning of prickle cell layer and thickening of outer cornified layers	184
Aroclor	Rabbit	Daily skin applications of 0.3, 0.6, and 0.9 g	High dose died before liver necrosis developed	Moderate doses: mottled liver, subacute yellow atrophy, fatty degeneration, and marked necrosis	Reddening; formation of small papules and blisters; finally desquamation of external epidermal layers	186
Aroclor 65% Cl	Rat	Inhalation of 0.57 mg/cubic meter for 16 hours for 37 to 134 days	0%	Pale and yellow; cell swelling; hyalin degeneration; potentiation of CCl_4 and C_2H_5OH toxicity		185

In recent studies of subacute toxicity, Allen and Norback (69) reported that rats fed 1000 ppm of Aroclors 1248, 1254, and 1262 died after 6-8 weeks due to widespread hepatic degeneration (188). After six weeks liver weights had increased from 2.8% of body weight in the controls to 9-10% of body weight in the treated animals. There was also marked atrophy of the thymus (188). Rats fed 1000 ppm of Aroclors 1254 and 1260 mostly died after 2-6 months with extensive liver damage (187); liver damage and a few deaths were observed also at 500 and 300 ppm (187, 189). In these cases the animals also suffered from severe pulmonary congestion and, in a few cases, from massive hemorrhages into the soft tissue or peritoneal cavity (187). Rats fed at 1000 ppm and 500 ppm of Kanechlors KC-500, 400 and 300 apparently had excess mortality within 25 weeks in conjunction with liver damage (190).

Mice fed 300 ppm of Aroclor 1254 for 6-11 months survived almost as well as controls but exhibited liver damage (191). Mice similarly survived well on a diet of 250 ppm KC-500, even in conjunction with BHC, but suffered liver damage (192). In comparative tests with three isomeric hexachlorobiphenyls (hexa-CBs) in male mice, the 28-day dietary LC_{50} was estimated to be over 300 ppm for 2,4,6,2',4',6'-hexa CB, 100-300 ppm for 2,4,5,2',4',5'-hexa CB, and only 86 ppm for 3,4,5,3',4',5'-hexa CB (193). In a subsequent test with 10 ppm of the last isomer, mice died in an average time of 39 days (193). The corresponding daily intake was about 2.1 mg/kg/day. In these experiments, atrophy of the thymus and spleen and porphyria were noted in addition to the liver effects

(193, 70 : Tables III.7.3 and III.7.4); these effects were especially marked with the exceptionally toxic isomer 3,4,5,3',4',5'-hexa CB.

Rhesus monkeys are considerably more sensitive to PCBs than rodents (69). Monkeys fed diets containing 100 and 300 ppm Aroclor 1248 developed facial edema, erythema, acne, and alopecia within 3 weeks. The lesions became progressively more severe with increased length of exposure. In addition, the animals developed anorexia, loss in weight, hypoproteinemia, hypolipidemia, and anemia. Within 3 months the majority of the monkeys were moribund. Necropsies of these animals revealed decided mucosal gastric hyperplasia with penetration of the glandular epithelium into the underlying submucosa (194). Numerous ulcerations of the hyperplastic gastric mucosa were also present (195). In addition, there was a decided hypertrophy of the liver.

Female rhesus monkeys fed 25 ppm Aroclor 1248 in the diet developed facial lesions similar to those observed in the animals receiving higher levels of PCBs (196). After two months on the PCB diet it was necessary to discontinue the exposure due to the severity of intoxication. One of the six experimental animals died 4 months after initial exposure to PCBs. Necropsy evaluation demonstrated severe gastric hyperplasia and ulceration, in addition to anemia, hypoproteinemia, bone marrow hypoplasia, and focal necrosis of the liver. The surviving adult female monkeys continued to be devoid of eyelashes and displayed facial acneform lesions 2 years following exposure to PCBs (69).

Rhesus monkeys appear to be even more sensitive to Aroclor 1242 than to Aroclor 1248. Although in the studies of Allen _et_ _al_.

Table III.7.3 (from ref. 70)

Summary of the biological effects of hexachlorobiphenyl isomers in mice

Chemical	Dose level (ppm)	Mortality	Body weight gain (% of control)	Liver effect	Porphyrin Accumulation	Thymus effect
3,4,5,3',4',5'-HCB	10	0/5	12	++	+	++
	100	3/5	-	+++	+++	+++
	300	5/5	-	-	-	+++
2,4,5,2',4',5'-HCB	100	0/5	91	+	-	-
	300	1/5	58	++	-	±
2,4,6,2',4',6'-HCB	100	0/5	85	+	-	-
	300	5/5	-	++	-	++

Table III.7.4 (from ref. 70)

Summary of major pathological changes in mice given different hexachlorobiphenyl isomers for 28 days

Chemical	Dose level (ppm)	Liver	Thymus	Spleen	Heart
3,4,5,3',4',5'-HCB	30	Marked; fatty metamorphosis, single-cell necrosis.	Marked; involution.	Moderate; depletion of lymphocytes.	NS[a]
2,4,5,2',4',5'-HCB	300	Slight; swelling of hepatocytes.	Slight; involution.	NS[a]	NS[a]
2,4,6,2',4',6'-HCB	300	Marked; fatty metamorphosis, single-cell necrosis.	Marked; involution.	Moderate; depletion of lymphocytes.	Moderate; cardiomyopathy.

[a] NS = not significant.

rhesus monkeys survived and even produced a few young on diets of 5 and 2.5 ppm Aroclor 1248 (69), in studies by McNulty (197) all animals exposed to dietary levels of 100, 30, 10 and even 3 ppm of Aroclor 1242 died. The monkeys fed 3 ppm died after 8 months. The symptoms of poisoning were similar to those induced by Aroclor 1248, including cystic eyelids, loss of sebaceous glands, atrophy of the thymus, proliferation of the smooth endoplasmic reticulum of the liver, hyperplasia and ulceration of the gastric mucosa, with penetration of the epithelium into the submucosa (197, 198).

In tests with individual isomers, 2,4,4'-trichlorophenyl and 2,4,5,2',5'-pentachlorobiphenyl proved relatively non-toxic, causing no histopathological changes in 90 days at 10 ppm; for comparison, Aroclor 1242 at 10 ppm causes clinically detectable changes within 30 days and outspoken disease at 60 days. However, 3,4,3',4'-tetrachlorobiphenyl was very toxic and killed the test animal in less than 60 days (197). These preliminary experiments indicate a high degree of structural specificity for the toxic effects of chlorobiphenyls in monkeys.

Mink also appear to be extremely sensitive to PCBs (199, 200). Mink fed on a diet containing 30 ppm PCBs (10 ppm each of Aroclors 1242, 1248 and 1254) all died between the beginning of the breeding season and the end of the whelping period. Clinical signs and lesions included anorexia, bloody feces, fatty infiltration and degeneration of the liver and kidneys, and haemorrhagic gastric ulcers (199). In subsequent tests with Aroclor 1254, reductions in weight gain and increased mortality were noted at dietary levels of 10 and 5 ppm; the effect of Aroclor 1254 was enhanced by simultaneous feeding of either DDT (10 ppm) or dieldrin (0.5 ppm) (Table III.7.5). All the mink fed 10 ppm of Aroclor 1254 and 0.5 ppm dieldrin died within 9 months (200). In a separate experiment, mink were fed on a diet containing beef from cows which had been fed with Aroclor 1254: the concentrations of PCBs in the total diet of two groups of mink were 3.57 and 0.64 ppm. All of the mink fed at the 3.57 ppm level died within 105 days, and 2 of 12 females fed at the 0.64 ppm level died at 122-129 days (201). Clinical signs of poisoning included yellowish discoloration of livers and blood in the gastrointestinal tract or intra-abdominal hemorrhage (see also Table III. 6.6). The PCBs fed in this experiment appeared to be markedly more toxic to mink than those in the first experiment, in which the animals even on the 10 ppm dose were still growing after 4 months (Table III.6.5). This suggests the possibility that the toxicity of the PCBs had been enhanced during metabolism and storage in the cows.

Another species which appears to be extremely sensitive to PCBs is the big brown bat Eptesicus fucus. When fed only 10 ppm Aroclor 1254 big brown bats grew more slowly than controls and some individuals died (72).

No studies appear to have been reported of the acute or subacute toxicity of Aroclor 1016 in any species of mammal.

Table III.7.5

Effect of Aroclor 1254, Alone or in Combination with Pesticides, on Body Weight Gains of Female Mink

			Body weight gain (gm) ± S.E. from initial wt.			
Dietary treatment	No. Mink	Initial body wt. (gm) (Aug. 25)	1 mo. (Sept. 24)	2 mo. (Oct. 25)	3 mo. (Nov. 25)	4 mo. (Dec. 22)
Basal diet (30% ocean fish mix; control)	6	1020	43 ± 17.4	203 ± 25.4	187 ± 46.2	210 ± 35.1
Basal diet plus 5 ppm PCB(1)	6	940	62 ± 22.4	153 ± 44.2	138 ± 42.7	128 ± 43.7
Basal diet plus 10 ppm PCB	6	923	43 ± 20.4	148 ± 32.0	132 ± 26.3	92 ± 35.1*
Basal diet plus 10 ppm PCB and 10 ppm DDT	6	1052	43 ± 14.5	57 ± 14.6**	28 ± 21.5**	-15 ± 35.4**
Basal diet plus 10 ppm PCB and 0.5 ppm dieldrin	6	940	108 ± 25.1	133 ± 26.7	115 ± 18.9	57 ± 24.4**

(1) Aroclor 1254.
*Significantly different ($P < 0.05$) from controls.
**Significantly different ($P < 0.01$) from controls.

Effect of Aroclor 1254, Fed Alone or in Combination with Pesticides, on Mortality and Reproduction of Mink (Aug. 25 to May 10)
(6 females and 1 male/group)

	% mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
Control	14	5	3	17	8	5.0
5 ppm PCB(1)	29	4	0	0	0	0
10 ppm PCB	71	1	0	0	0	0
10 ppm PCB + 10 ppm DDT	43	4	0	0	0	0
10 ppm PCB + 0.5 ppm dieldrin	100	0	0	0	0	0

(1) Aroclor 1254.

III.8. Toxicity of PCDFs in Mammals and the Role of PCDFs in the Toxicity of Commercial PCBs

Preliminary studies of the toxicity of 2,3,7,8-tetrachlorodibenzofuran (TCDF) in mammals have been reported by Moore et al. (71) and Goldstein et al. (146). In guinea pigs, the single-dose oral LD_{50} is between 5 and 10 µg/kg (Table III.8.1). The bottom of the table shows, for comparative purposes, that the LD_{50} of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (2 µg/kg) compares quite closely with the TCDF value. TCDF toxicity in guinea pigs is one of a progressive weight loss followed by depression at least 24 to 36 hours preceding death. As was observed in the chicken toxicity studies, there was severe diminution in size of thymus and spleen. Similarly, this finding was found to correlate with a lymphocyte depletion in the periarterialor lymphocytic sheaths and follicles of the spleen; or severe atrophy of the thymus cortex. Although the numbers of animals evaluated were small, the trend of changes present in the bladder, adrenal, kidney, and liver were similar to that previously associated with TCDD toxicity (202). In contrast, TCDF appears to be much less toxic to rats and mice than to guinea pigs (71). A single experiment in which TCDF was administered to rats at doses up to 1,000 µg/kg failed to show any toxic effects. These rats were terminated 4 weeks after dosing. Histopathologic evaluation of tissues from the rats failed to reveal any changes that could be associated with tetrachlorodibenzofuran toxicity.

Table III.8.2 summarizes the TCDF toxicity findings in male mice. Per os dose levels up to and including 6,000 µg/kg in

Table III.8.2 (from ref. 71)

2,3,7,8-tetrachlorodibenzofuran toxicity in male C57 BL/6 mice[a]

Dose:	0 µg/kg	6,000 µg/kg
Mortality:	None	None
Body weight:	Normal gain	Sl. depression 2nd-5th day
Liver/body weight:	1.513 + 0.05	1.728 + 0.04
Ratio:	5.76 + 0.05	6.86 + 0.06
Thymus/body weight:	0.045 + 0.002	0.017 + 0.001
Ratio:	0.19 + 0.01	0.06 + 0.007

[a] Administered subcutaneously to 6-week-old mice. Animals sacrificed 30 days post-administration. "No effects" per, os, at 6,000, 4,000, 2,500, 1,500, 1,200, 1,000, 800, 600, and 400 µg/kg. LD_{50} 2,3,7,8-TCDD = 200-250 µg/kg.

Table III.8.1 (from ref. 71)

2.3,7,8-tetrachlorodibenzofuran toxicity in Hartley guinea pigs

Per os dose (µg/kg)	Mortality	Mean time to death
80	5/5	10.8 days
40	5/5	11.6
20	5/5	12.2
15	6/6	12.0
10	9/11	15.5
5	3/11	18.0
0	0/11	-0-

LD_{50} 2,3,7,8-TCDD = 2.0 µg/kg; MTD = 20.6 days.

a single dose failed to produce any observable effects during the ensuing 28 to 30 days. Histopathologic examinations of these animals failed to reveal any effects on thymus, spleen, or other organs, save for the liver. Here, a mild effect with the suggestions of a mild toxicity was observed in mice that received 6,000 µg/kg _per os_. A definite toxic response was observed in mice that received a 6,000 µg/kg dose administered subcutaneously. Although mortality was not observed, there was a depression in body weight gain during the second to fifth day following dosing. Organ weights recorded at time of necropsy 30 days after the dose had been administered showed an increase in the liver weight as well as liver to body weight ratio.

A more striking finding was the reduction in thymus weight with a concurrent reduction in thymus to body weight ratio. Histopathologic evaluations of tissues from these animals confirmed that the reduction in thymus weight was due to loss of lymphocytic elements. Evaluation of the liver evidenced a clear, but moderate, toxic response characterized by focal areas of single- cell necrosis and pleomorphism. The mouse studies show that there is at least a 30-fold diminution in TCDF toxicity when compared to TCDD. It further indicates, however, that the pattern of the toxicity will mimic that which is observed with TCDD (203).

Although 2,3,7,8-TCDF is the only CDF isomer whose toxicity has been investigated to date, it is expected to be the most toxic CDF, by analogy with the structurally similar 2,3,7,8-TCDD (148). It is important to note that 2,3,7,8-TCDF is one of the CDFs that has been identified in Aroclors 1248 and 1254 (36).

The toxic effects of TCDF in mice are similar to those of 3,4,5,3',4',5'-hexa-CB (Tables III.7.3, III.7.4), but there are some important differences. The hexa-CB had a proportionately greater effect on the thymus and liver, and induced p-nitrophenyl glucuronyl transferase and ALA-synthetase, causing porphyria (146); as in the case of chicks (Figure III.6.4, Table III.6.7), TCDF did not have these effects in mice, even at comparable dose-levels (71, 146).

In a comparative study, Vos and Beems (204) found differences in toxicity between Aroclor 1260, Clophen A60 and Phenoclor DP-6 when applied to the skin of rabbits (118 mg PCB per rabbit 27 times in 38 days). Clophen A60 and Phenoclor DP-6 caused mortality (3/4 and 1/4 respectively) and severe skin lesions, whereas Aroclor 1260 caused no mortality and only slight skin lesions. All three mixtures caused liver damage, abdominal and pericardial edema, kidney lesions, and porphyria, but the liver damage was less severe in the Aroclor 1260-treated animals (Table III.8.3). Since PCDFs were known to cause skin lesions of this type, Vos attributed the difference in toxicity primarily to the PCDFs in the Clophen and Phenoclor mixtures (204, 129). However, in a subsequent experiment (205) both Aroclor 1260 and 2,4,5,2',4',5'-hexa-CB* caused liver injury, porphyria

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* Some synthesized samples of 2,4,5,2',4',5'-hexa-CB are now known to be contaminated with 2,3,7,8-TCDF (206), but this does not appear to have been the case for the sample used in ref. 205.

Table III.8.3 (from ref. 204)

PATHOLOGIC FINDINGS IN LIVERS OF RABBITS TREATED WITH PCB FOR 38 DAYS, AND OF CONTROL ANIMALS[a]

	Phenoclor				Clophen				Aroclor				Control			
			b		*b*		*b*	*b*								
Fatty degeneration	2	2	1	1	1	1	1	—	—	—	1	2	—	—	—	—
Centrolobular loss of glycogen	—	1	2	1	2	1	1	2	—	—	1	—	—	—	—	—
Centrolobular degeneration	1	2	—	1	1	2	1	2	—	1	1	1	—	—	—	—
Focal hydropic degeneration	—	1	—	—	—	2	2	—	—	1	—	2	—	—	—	—
Focal necrosis	—	1	—	—	2	2	2	2	—	—	1	2	—	—	—	—
Centrolobular liver cell atrophy	2	1	1	2	2	1	2	1	—	—	—	1	—	—	—	—
Enlargement of nuclei	2	1	—	1	2	2	2	2	1	—	1	2	—	—	—	1
Cytoplasmic hyalin degeneration	1	—	1	2	2	1	2	2	—	—	—	1	—	—	—	—
Ceroid pigment in Kupffer cells	—	1	—	2	1	1	1	1	—	2	1	2	—	—	—	—
Periportal fibrosis	2	—	2	1	1	1	2	2	1	—	1	1	—	—	—	—

[a] Severity of lesion: 1 = slight, 2 = marked.
[b] Animals that died during the experiment.

and slight skin lesions. Vos (129) concluded that both PCDFs and PCBs cause liver damage and skin effects, but that PCBs are the primary cause of porphyria (Table III.8.4). This conclusion has been confirmed by subsequent experimentation (70, 71, 146, 147, 193), but several experiments have suggested further that PCBs play some role in the induction of edema and hydropericardium also (Section III.6.2 above, 204).

McNulty (197) has discussed the possibility that PCDFs might be responsible for the extreme toxicity of PCBs in rhesus monkeys. In a preliminary test, 2,3,7,8-TCDD was lethal to a rhesus monkey after feeding for two months at 2 ppb in the diet -- i.e., it was about 5,000 times as toxic as Aroclor 1242 under the same chronic feeding conditions. TCDD is usually 4 to 10 times as toxic as TCDF (71, 129): hence it is unlikely that TCDF will prove to be more than 1,000 times as toxic as Aroclor 1242. Accordingly it is very unlikely that the presence of PCDFs at 1 or 2 ppm in Aroclor 1242 could account for its toxicity to monkeys (197). McNulty suggested the possibility that PCDFs might be produced by metabolism in monkeys (197). The relative roles of PCBs and PCDFs in causing skin lesions in humans will be discussed further below.

In summary, 2,3,7,8-TCDF is extraordinarily toxic to guinea pigs and to chickens, but not to mice or rats. There are both parallels and differences between the toxic effects of PCBs and PCDFs. Some, but not all, of the toxic effects of PCB mixtures can be attributed to contamination with PCDFs. Since PCDFs are present in

Table III.8.4 (from ref. 129)

Probable Contribution of Polychlorinated Dibenzofuran (PCF) and Pure Polychlorinated Biphenyl (PCB) in the Toxicity of Crude PCB Mixtures.

	Chlor-acne	Edema formation	Liver damage	Hepatic porphyria
Polychlorinated Dibenzofuran	++	++	++	–
Polychlorinated Biphenyl	+	–	+	++

commercial mixtures (Section II.5.5) and appear to be formed from them in the environment (Section II.8.3), in service (Section II.8.4), and by metabolism (Appendix E), it is difficult and probably academic to attempt to distinguish their effects completely in weighing the environmental hazards posed by PCBs.

III.9 Chronic Effects of PCBs in Mammals and Effects on Reproduction

This section summarizes the results of long-term chronic toxicity tests with PCBs in mammals and the gross effects observed, including effects on reproduction. Discussion of biochemical effects is reserved to Sections III.10 and III.11 and discussion of carcinogenic effects to Section III.14.

III.9.1 Effects on rats

In a study reported by Linder *et al*. (179), Sherman rats were exposed for two generations to dietary levels of 1, 5, 20 and 100 ppm Aroclor 1254, and 5, 20 and 100 ppm Aroclor 1260. Exposure of the F_o generation started at 3-4 weeks of age and continued through mating, gestation and lactation. Each generation was mated twice. Rats exposed to dietary levels of 20 ppm (1.5 mg/kg/day) or more had fewer pups per litter than controls in the F_{1b} and F_2 generations. The 100 ppm exposure level of Aroclor 1254 increased mortality in the F_{1b} offspring and markedly decreased mating performance of the F_{1b} adults (Table III.9.1). The 500 ppm dietary level of Aroclor 1260 reduced litter size and decreased survival in the F_1 litters. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no

Table III.9.1 (from ref. 179)

Reproduction and pup survival in groups of rats fed Aroclor 1254

Generation	Dietary level (ppm)	Parental exposure (days)†	No. of females mated	No. of litters		Litter sizes§		Total pups/group				Survival (%)	Mean body weight at weaning (g)
								Born (found)		Alive at			
				Born‡	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
F_{1a}	0‖	67	10	8	7	11·1	10·6	3	89	86	85	95·5	39·2
	100‖	67	10	9	8	9·4	8·1	6	85	78	73	85·9	31·4
	500‖	67	10	4 (2)	0	4·0*	—	5	8	0	0	0	—
	0	62	20	17	17	12·4	11·8	0	211	205	201	95·3	38·7
	20	62	20	19	19	11·7	11·5	3	222	220	219	98·6	38·3
	100	62	20	19	19	10·7*	10·3	10	204	199	196	96·1	32·9
	0	67	20	18	18	11·2	11·1	2	202	200	200	99·0	38·5
	1	67	20	16	15	9·2	9·1	3	147	145	145	98·6	42·3
	5	67	20	17	17	10·8	10·8	1	184	184	183	99·5	42·1
F_{1b}	0‖	186	10	7	7	11·6	11·6	1	81	81	81	100	37·9
	100‖	186	10	8	6	11·8	8·0	0	94	74	64	68·1**	27·9
	0	188	20	17	17	12·3	11·1	2	209	194	189	90·4	37·1
	20	188	20	18	18	10·4*	10·1	1	188	187	181	96·3	39·0
	100	188	20	20	19	9·5**	7·0	6	189	164	139	73·6	32·1
	0	201	20	18	18	12·3	11·0	6	222	211	198	89·2	40·7
	1	201	20	18	17	11·1	9·8	3	200	194	176	88·0	38·0
	5	201	20	20	19	11·1	10·2	2	222	218	203	91·4	38·9
F_{2a}	0	129	20	18	18	12·5	12·2	0	225	221	220	97·8	35·5
	20	129	20	17	17	10·6*	10·1	5	181	174	171	94·5	36·8
	100	129	20	7 (2)	4	7·2**	5·6	7	36	29	28	77·8**	35·2
	0	125	20	19	19	11·7	11·5	3	223	220	218	97·8	37·4
	1	125	20	15	15	11·7	11·5	0	175	174	173	98·9	37·2
	5	125	19	17	17	12·1	11·7	1	205	199	198	96·6	35·7
F_{2b}	0	274	20	17	16	12·7	11·3	4	216	201	191	88·4	31·9
	20	274	20	12	12	9·6**	8·5	6	115	103	102	88·7	36·1
	100	274	20	4 (2)	2	3·5**	3·5	7	7	7	7	100·0	38·3

† Conception to mating for the parents of the F_{2a} and F_{2b} generations.

‡ Numbers in parentheses indicate numbers of litters in which no live offspring were found.

§ Number of live offspring/live litter born.

‖ One-generation study.

Values marked with asterisks differ significantly from the control value: * $P < 0{\cdot}05$; ** $P < 0{\cdot}005$.

effects on reproduction. Liver weights were markedly increased in 21-day-old male weanlings at the 1 ppm level of Aroclor 1254 and in both sexes in both generations at 5 ppm or higher levels of Aroclor 1260 (Table III.9.2).

In this study rats were killed when their offspring were weaned (after 180-330 days' exposure) (179). In this and in earlier studies in which rats were exposed to Aroclor 1254 and 1260 for periods of 6 to 8 months (187, 189), the principal pathological lesions observed were in the liver. These included hypotrophy, brown pigment in Kupffer cells, adenofibrosis and hyperplastic nodules (179, 187, 189). Adenofibrosis and nodules were observed principally at the higher levels of exposure and were most frequent in the second generation animals exposed to Aroclor 1254 (Table III.9.3). Liver nodules were even seen in F_{1b} rats exposed to 20 ppm Aroclor 1254 (ibid.). For further discussion see below under Carcinogenesis and Appendix D.

In another three-generation reproduction study in rats involving exposure to Aroclors 1242, 1254, and 1260, reproduction was unaffected in the first generation, but was adversely affected at dietary levels of 10 and 100 ppm of all three Aroclors in the second and third generations (68). No adverse effects were reported at 1 ppm. The cause of the adverse effects was a decrease in mating indices and in the incidence of pregnancy (68). Other aspects of reproduction are said to have been unaffected, but no details have been given, and in earlier reports on this study decreased survival of offspring was reported at 100 ppm of Aroclors 1242 and 1254 (1, p. 142). In a concomitant 2-year study of rats exposed to the same three Aroclor

Table III.9.2 (from ref. 179)

. Mean liver weights of 21-day-old rats from parents fed Aroclor 1254 or 1260

Aroclor	Generation	Dietary level (ppm)	Liver weight			
			Males		Females	
			g	% of body weight	g	% of body weight
1254	F_{1a}	0	1·40	3·69	1·38	3·83
		1	1·81*	4·01*	1·63	3·91
		5	1·92*	4·34*	1·86*	4·43*
	F_{1b}	0	1·58	3·69	1·58	3·84
		1	1·54	3·94*	1·49	3·92
		5	1·77	4·63*	1·72	4·75*
	F_{2a}	0	1·37	3·81	1·28	3·74
		20	2·26*	5·78*	2·23*	6·08*
		100	2·63*	6·97*	2·67*	7·10*
	F_{2a}	0	1·63	3·78	1·42	3·63
		1	1·49	3·74	1·36	3·69
		5	1·56	4·23*	1·38	4·09*
1260	F_{1a}	0	1·54	3·66	1·57	3·92
		5	1·73	4·18*	1·70	4·39*
	F_{1b}	0	1·59	3·73	1·55	3·93
		20	2·06*	5·04*	2·14*	5·18*
		100	2·59*	6·33*	2·59*	6·48*
	F_{1b}	0	1·35	3·59	1·29	3·70
		5	1·58	4·01*	1·52*	4·07*
	F_{2a}	0	1·65	3·82	1·64	4·02
		20	2·06*	5·21*	1·94	5·06*
		100	2·50*	6·12*	2·49*	6·85*
	F_{2a}	0	1·41	3·85	1·36	3·87
		5	1·66*	4·21*	1·60*	4·25*

Values marked with an asterisk differ significantly from the control value: * $P < 0·05$.

Table III.9.3 (from ref. 179)

Body weight, liver weight and liver pathology of F_0 and F_{1b} adult rats fed Aroclor 1254 or 1260

Generation	Sex	Exposure (days)	Dietary level (ppm)	Terminal body weight (g)	Liver weight		No. of livers examined	Liver pathology (no. of livers affected)						
					g	% of body weight		Enlarged hepatocytes	Inclusions	Foamy cytoplasm	Pigment	Fibrous strands	Adenofibrosis	Nodules
Aroclor 1254														
F_0	M	310	0	601	15·22	2·53	10	0	0	0	0	0	0	0
			1	602	15·88	2·60	10	0	0	0	0	0	0	0
			5	614	15·37	2·51	10	0	0	1	2	0	0	0
	F	313	0	370	10·34	2·80	10	0	0	0	1	0	0	0
			1	358	10·14	2·84	10	1	0	0	4	0	0	0
			5	362	10·66	2·94	10	4	1	0	3	0	0	0
F_{1b}	M	328	0	588	14·51	2·47	10	0	0	0	0	0	0	0
			20	602	16·11*	2·67*	8	8	3	7	1	0	0	0
			100	554	19·43*	3·54*	10	10	5	7	4	2	3	1
	F	328	0	349	9·92	2·85	10	0	0	0	0	0	0	0
			20	340	9·41	2·78	7	7	2	5	7	0	0	3
			100	300*	10·53	3·60*	10†	10	2	8	9	3	5	7
F_{1b}	M	190	0	475	11·13	2·34	10	0	0	0	0	0	0	0
			1	453	11·14	2·44	10	0	0	0	0	1	0	0
			5	495	12·19	2·46	10	2	0	2	0	1	0	0
	F	190	0	287	8·27	2·87	10	0	0	0	0	0	0	0
			1	294	8·44	2·86	10	1	1	0	0	4	0	0
			5	299	8·92	2·98	10	1	0	0	0	1	0	0
Aroclor 1260														
F_0	M	250	0	563	14·45	2·57	10	1	0	0	0	0	0	0
			5	567	15·06	2·65	10	3	0	2	0	0	0	0
	F	250	0	322	10·09	3·13	10	0	0	0	0	0	0	0
			5	331	10·80	3·25	10	1	0	0	0	0	0	0
F_{1b}	M	179	0	472	12·68	2·68	10	0	0	0	0	0	0	0
			20	494	14·72*	2·99*	10	8	5	4	0	0	0	0
			100	509	16·63*	3·27*	10	10	10	3	0	0	0	1
	F	179	0	297	9·66	3·27	10	0	0	0	0	0	0	0
			20	304	10·30	3·40	10	6	0	0	0	0	0	0
			100	304	11·14*	3·70*	10	9	0	1	5	2	1	2
F_{1b}	M	217	0	492	11·97	2·43	10	0	0	0	0	0	0	0
			5	505	13·16	2·60*	10	7	2	2	1	0	0	0
	F	217	0	279	7·93	2·84	10	0	0	0	0	0	0	0
			5	286	8·49	2·96	10	2	0	0	0	0	0	0

† Necrosis in two livers.

Values marked with an asterisk differ significantly from the control value: * P 0.05.

mixtures, the most noteworthy effects were increases in liver weight and pathological changes in the liver (68, 207: for further discussion see below under Carcinogenesis and Appendix D).

Sherman rats exposed to 100 ppm of Aroclors 1016 and 1242 for 4-10 months also showed a variety of microscopic changes in their livers (208). These changes were somewhat less severe than those observed in animals fed 100 ppm of Aroclors 1254 and 1260 (Tables III. 9.4, III.9.5), in that the animals fed Aroclors 1016 and 1242 did not show the lipid accumulation pronounced in the other groups. The changes were slightly, but not markedly, less severe in the rats fed Aroclor 1016 than in those fed Aroclor 1242 (Table III.9.4).

In a short-term (90-day) oral toxicity study with Aroclor 1016, liver weights were significantly increased at dietary exposure levels of 300 ppm, but not at 100 or 30 ppm (209). The study was too short, however, to provide a useful comparison with that in ref. 207, in which no changes in liver weight were reported in rats exposed to 100 ppm Aroclor 1242 for 3 months. However, in another study, the liver weights of rats were markedly increased by oral dosage of only 1 mg/kg (equivalent to about 15 ppm in the diet) for only 21 days (Table III.10.5): the effects of Aroclors 1016 and 1242 were similar (243). In another study, rats exposed to 100 ppm Aroclor 1242 for 2 months showed a significant increase in liver weight and a marked decline in liver vitamin A (166). Aroclor 1242 also had estrogenic effects in female rats (166), and dietary exposure to 30 or 100 ppm Aroclor 1016 markedly reduced gonad weights in female rats (209).

Table III.9.4 (ref. 208)

—Incidence of Microscopic Changes Observed in Livers of Rats Fed Aroclor 1242 or 1016

Length of Exposure	Enlarged Hepatocytes		Vacuolated Cytoplasm		Inclusions		Brown Pigment		Foamy Cytoplasm		Foci of Necrosis Hemorrhage	
	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016
4	2/4	4/4	...	1/4	...	...	...	...	...	...	...	1/4
6	4/4	4/4	3/4	1/4	3/4	3/4	...	...	...	...	...	...
8	4/4	3/4	3/4	...	3/4	...	2/4	...	...	...	1/4	...
10	4/4	4/4	4/4	1/4	1/4	...	2/4	...	2/4	...	...	...
6 mo exp 2 mo rec	2/4	1/4	3/4	1/4	1/4	...	...	...	...	...	...	...
6 mo exp 4 mo rec	3/3	3/3	1/3	1/3	1/3	1/3	...	...	...	...	...	...
6 mo exp* 6 mo rec	3/4	...	...	...	2/4	...	...	...	...	...	...	...

* Only rats fed Aroclor 1242 were examined microscopically.

Table III.9.5 (ref. 187)

—Light Microscopic Findings in the Liver of Rats Fed Aroclor 1260*

Dietary Level (ppm)	Sex	Enlarged Hepatocytes	Inclusions in Cytoplasm	Adenofibrosis	Increased Lipid	Foamy Cytoplasm	Pigment
0	M	...	...	...	...	...	...
20	M	10	9	...	...	3	...
100	M	10	9	...	10	...	...
500	M	10	4	...	10	7	1
1,000	M	10	6	2	10	9	2
0	F	...	...	...	...	...	...
20	F	3	...	...	...	...	...
100	F	7	2	1	10	...	1
500†	F	9	5	1	9	8	6
1,000‡	F	6	...	4	7	4	2

* In their diet for eight months; ten rats/group. † Only nine animals were studied. ‡ Only seven animals were studiec

. —Light Microscopic Findings in the Liver of Rats Fed Aroclor 1254*

Dietary Level (ppm)	Sex	Enlarged Hepatocytes	Inclusions in Cytoplasm	Adenofibrosis	Increased Lipid	Foamy Cytoplasm	Pigment
0	M	...	...	...	...	...	...
20	M	7	1	...	...	1	...
100	M	10	7	1	10	10	3
500	M	10	2	10	10	4	2
0	F	...	...	...	...	...	...
20	F	...	...	...	...	...	1
100	F	10	7	7	10	9	10
500†	F	9	2	9	9	2	7

* In their diet for eight months; ten rats/group.
† Only nine tested since one rat that died showed too much autolysis.

III.9.2 Effects on mice

In chronic feeding studies with mice, the most noteworthy effects were on the liver (see below under Carcinogenesis and Appendix D). Daily dosage of mice with 1 ml KC-400 caused effects on the skin (loss of hair, erosion and ulceration after 3 months) in addition to enlargement of the liver and other pathologic changes (210).

No chronic studies of effects on reproduction have been reported, but a single dose of 20 mg/kg Clophen A60 lengthened oestrous cycles in mice (211).

III.9.3 Effects on dogs

A two-year chronic feeding study was conducted in beagle dogs exposed to 1, 10, and 100 ppm of Aroclors 1242, 1254 and 1260 in the diet (68, 212). Four dogs of each sex were included in each of the 9 feeding groups and in a tenth control group; the dogs were 4-7 months old at the start of exposure. Six dogs died during the experiment: of these three females died from chronic peritonitis (one on 100 ppm Aroclor 1242, one on 10 ppm Aroclor 1254, and one on 100 ppm Aroclor 1260). Table III.9.6 summarizes the principal findings at the end of the two-year exposure period: there were dose-related increases in liver weights, serum alkaline phosphatase levels, and leukocyte counts. The most noteworthy findings on gross pathologic observation were "numerous pinpoint nodules" in the stomachs of dogs in all treatment groups (except the 1 ppm Aroclor 1242 group). These nodules were observed in 22 of the 66 treated dogs,

Table III.9.6. Effects in dogs after two-year oral exposure to PCBs (from data in ref. 212)

	Leukocyte counts (thousands/mm^3)	Serum alkaline phosphatase KA units/100 ml	Liver weight, % of body weight	Pinpoint nodules in stomach
Controls (8)	11.45	5.22	3.51	0/8
Aroclor 1242:				
1 ppm (8)	12.44	4.48	3.26	0/8
10 ppm (7)	13.46*	5.93	3.62	3/7
100 ppm (7)	19.93***	5.91	3.87	4/7
Aroclor 1254:				
1 ppm (8)	14.26**	5.62	3.52	3/8
10 ppm (7)	14.19**	7.49	3.64	1/7
100 ppm (7)	20.59***	9.64**	4.11*	5/7
Aroclor 1260:				
1 ppm (8)	14.28	5.77	3.77	2/8
10 ppm (8)	14.69**	4.98	3.73	2/8
100 ppm (6)	12.11	30.22***	5.03**	2/6

*, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$; significant differences from controls.

versus none in the 8 controls. No histopathological diagnoses of these lesions were included in the original report (212), but in 1975 it was reported that re-examination of the slides had shown evidence of "gastrointestinal inflammatory lesions and ulcerations which appeared similar to those reported by Allen (69) in rhesus monkeys".* Six of the dogs with stomach lesions, and two of the others, also had inflamed kidneys. The stomach lesions were equally prominent in dogs fed all three Aroclors, but the effect on liver weight was greatest in those fed Aroclor 1260 (Table III.9.6).

III.9.3 Effects on Non-human Primates

Rhesus monkeys are extremely susceptible to the effects of PCBs (69). Female monkeys fed 25 ppm Aroclor 1248 in the diet developed severe facial lesions and alopecia and had to be removed from the contaminated diet after 2 months. The five surviving females were still devoid of eyelashes and had facial acneform lesions two years later. Although several of these females conceived, all but one appeared to experience either fetal resorption or abortion. The one infant born was underweight (375 g versus 544 g) and had high levels of PCBs in the fat (28 ppm) and adrenals (24 ppm) (196).

Female monkeys given 2.5 and 5.0 ppm Aroclor 1248 in their diets developed facial edema, swollen eyelids, erythema, loss of hair, and acne within 2 months (213). By the fourth month, irregularities in the menstrual cycles and an increased level of urinary ketosteroids were recorded (214). Following 6 months of PCB exposure the female monkeys were bred to control males. Six of eight animals on the 5.0 ppm diet conceived (Table III.9.7). The remaining two were bred on five separate occasions without conceiving. Four of the six females experienced abortion early in gestation. Eight of eight of the 2.5 ppm PCB-fed animals conceived; however, only five were able to carry their infants to term. As was the case with infants of animals given the higher levels of PCBs, all the infants were small and at birth their skin contained detectable levels of PCBs.

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* This statement, made at the National Conference (9), was deleted from Calandra's published paper (68), which states that "No remarkable histopathologic changes were found".

The infants were permitted to nurse their mothers for 4 months.* Within 2 months focal areas of hyperpigmentation, swollen lips and eyelids, loss of eyelashes, and acneform lesions of the face developed. The skin of these infants showed a decided increase in the PCB level over this period. Within 4 months, 3 of the 6 infants died due to PCB intoxication. After weaning, the remaining three have shown improvement of the skin lesions during the 4-month period.

Earlier studies by this group have shown that infant rhesus monkeys are susceptible to other effects of PCBs. Month-old rhesus monkeys dosed with 35 mg/kg Aroclor 1248 for only four weeks suffered, in addition to skin lesions, atrophy of the thymus, edema and hyperplasia of the gastric mucosa, enlargement of the liver with proliferation of the smooth endoplasmic reticulum, and an increase in the RNA/DNA ratio in liver (214). Adult rhesus monkeys displayed hyperplasia and ulceration of the gastric mucosa after a single dose of 1.5 g Aroclor 1248 (215).

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* The mothers' milk contained 0.15-0.40 ppm of PCBs (69).

Table III.9.7 (from ref. 69)

Modification in reproduction in primates that were exposed to Aroclor 1248 in the diet

	Control	2.5 ppm	5.0 ppm
Total impregnated (no./no. animals)	12/12	8/8	6/8
Resorptions or abortions (no./no. animals)	0/12	3/8	4/8
Stillborn (no./no. animals)	0/12	0/8	1/8
Normal births (no./no. animals)	12/12	5/8	1/8

Male monkeys appear to be less susceptible than females to reproductive dysfunction by PCBs (69). Four adult male rhesus monkeys were also exposed to a diet containing 5.0 ppm Aroclor 1248 for 17 months (average total intake of PCBs 460 mg). They began to develop a slight periorbital edema after 6 months of exposure; however, it was much less severe than in the female monkeys receiving a similar level of PCB. The morphological features and viability of the spermatozoa as well as the ability to fertilize control female rhesus monkeys was unaffected during the initial 12 months of PCB exposure. Subsequently one of the four males lost weight and developed alopecia, acne, periorbital edema and decreased libido. A testicular biopsy of this animal showed a decided hypoactivity of the seminiferous tubules. There was an absence of mature spermatozoa and a predominance of Sertoli cells of the tubules. The remaining three males have remained healthy and sexually active (69).

III.9.4 Effects on Mink

Mink also appear extremely sensitive to the effects of PCBs. In tests with Aroclor 1254, most females died and none produced young when fed diets containing 15 or 10 ppm (199, 200). In one test at 5 ppm no young were produced, and in a second test only 3 young were born alive from 12 exposed females (Table III.9.8). Reproduction was also reduced at 1 ppm Aroclor 1254, because one female died and two others produced no young (Table III.9.8). In another experiment in which mink were fed a diet containing 0.64 ppm of PCB residues in beef resulting from feeding PCBs to cows, only one of ten exposed

Table III.9.8 (from ref. 200)

Effect of Aroclor 1254 Fed Singly and in Combination with Hydrocarbon on Reproductive Parameters (Jan. 1 to May 10) (12 females and 4 males/group)

	% mortality	Females alive	Females whelped	Kits born		Kits/female alive
				Alive	Dead	
Control (ocean fish)	6	11	11	56	10	6.0
Raw coho salmon	19	11	0	0	0	0
Cooked coho salmon	6	12	0	0	0	0
1 ppm PCB(1)	5*	10	8	35	8	4.3
5 ppm PCB	0	12	3	3	6	0.8
15 ppm PCB	31	8	0	0	0	0
5 ppm PCB + 6 ppm DDT	0	12	4	5	6	0.9
5 ppm PCB + 6 ppm DDT + 0.2 ppm dieldrin	19	10	5	15	7	2.2

(1) Aroclor 1254.

* One female escaped.

females produced a litter: three kits were born to this female but they died during their first day of life (201). Thus, as already noted in Section III.7 above, the toxicity of the PCBs appears to have been enhanced during their metabolism and storage in the cows.

III.9.5 Effects on Swine

Hansen et al. (216) reported an experiment in which sows were fed a diet containing 20 ppm Aroclor 1242 from three weeks prior to conception throughout gestation and nursing. One of five treated sows failed to conceive and the other four produced significantly fewer live young than controls, due to an excess of stillbirths and fetal deaths (Table III.9.9). Treated sows had enlarged livers and their offspring had unusually small spleens and thyroids (Table III.9.10). The principal lesions observed in both sows and offspring were erosions in the gastric mucosa. A number of the treated offspring, but no controls, died from chronic septicemia, suggesting that the exposure to PCBs had increased their susceptibility to infection. These adverse effects were associated with residues of only 4-20 ppm in the fat of the sows, and less than 2 ppm in their milk (214).

III.9.6 Effects on rabbits

Oral administration of a single dose of 12.5, 25, or 50 mg/kg of Aroclor 1254 to pregnant rabbits had embryotoxic effects, but single doses of 10 and 1 mg/kg did not (217, 218).

Table III.9.9 (from ref. 216)

Farrowing Records and Polychlorinated Biphenyl (PCB) Residues of Sows Fed PCB Mixture in Ration and of Nontreated Control Sows

Farrowing weight (kg)	Total PCB (ppm)		Offspring			
			Live			
	Blood	Fat	No.	Mean body weight (kg)	Still-born	Fetal deaths*
CONTROL SOWS						
268	0.001	0.1	13	1.1	5**	0
277	0.001	0.1	12	1.5	0	0
256	0.001	0.1	10	1.6	1	0
250	0.001	0.1	11	1.5	0	0
254	0.001	0.1	11	1.5	1	1
TREATED SOWS						
268	0.086	4.1	11	1.2	2	0
264	0.192	11.8	10	1.5	1	1
270	0.363	19.8	6	1.4	1	8
259	.....	8.1	5	1.5	0	1
286†	0.337	11.7	.....	.....	.....	.....

* Mummified, fetuses (2.5 cm to near term) from examination of placenta. ** Last 5 in litter of 18; delivered 28 to 36 hours after 1st pig. † Fed control ration for 5 weeks before slaughter; this sow did not conceive.

Table III.9.10 (from ref. 216)

Organ Weight to Body Weight Ratios for Sows Given PCB Mixture in the Feed, for Nontreated Control Sows, and for Their Offspring

Organ	Organ weight to body weight ratio (mean × 10^2 ± SD)			
	Sows		Offspring	
	Control	Treated	Control	Treated
Liver	11.37 ± 0.08	14.50 ± 1.68	24.23 ± 0.97	25.20 ± 1.70
Kidney	1.71 ± 0.33	2.02 ± 0.40	5.08 ± 0.35	4.39 ± 0.70
Spleen	1.09 ± 0.05	1.19 ± 0.19	1.72 ± 0.24	1.27 ± 0.13
Heart	2.64 ± 0.40	2.53 ± 0.22	4.82 ± 0.51	4.40 ± 0.35
Brain	0.70 ± 0.40	0.57 ± 0.06	3.55 ± 0.44	3.70 ± 0.34
Thyroid gland	0.055 (n = 1)	0.050 ± 0.003	0.111 ± 0.015	0.076 ± 0.008
Adrenal gland	0.070 ± 0.002	0.086 ± 0.008	0.080 ± 0.010	0.068 ± 0.012

n = Number of sows; SD = standard deviation.

III.10. Enzyme Induction and Other Effects on the Liver

Some of the most general and significant sublethal effects of PCBs in mammals are on the liver and on hepatic enzymes. This section reviews these effects, with the exception of chemical porphyria (Section III.11) and carcinogenesis (Section III.14).

III.10.1. Effects on liver weight

An increase in liver weight and/or in liver-to-body weight ratio has been noted in many studies. Litterst et al. (219) observed increased liver-to-body weight ratios in rats fed 50 and 500 ppm of Aroclors 1242, 1248, 1254 and 1260 for only 4 weeks (Table III.10.1). Similar increases in liver weight have been noted in long-term feeding studies with these mixtures in rats (69, 166, 179, 207). In general the effects were greater with the more chlorinated mixtures (Table III.10.1, 207), but in an experiment with Aroclor 1016 marked effects were seen in only 21 days after oral dosage of only 1 mg/kg/day (equivalent to about 12 ppm in the diet) (Table III.10.5). In addition, effects were noted at much lower dietary levels in offspring of treated rats (exposed during gestation and prior to weaning): significant increases in liver weight were noted in weanling rats after maternal exposure to only 5 ppm Aroclor 1260 and only 1 ppm Aroclor 1254 (179: Table III.9.2).

Increase in liver weight has been noted as an effect of PCBs in many other species (188, 193, 129, 210, 212, 214, 216), and is also a toxic effect of PCDFs (129, 71).

Table III.10.1 (ref. 219)

EFFECT OF PCB TREATMENT ON FINAL LIVER WEIGHTS AND ON LIVER : BODY WEIGHT RATIO IN MALE OSBORNE-MENDEL RATS[a]

Aroclor No.[b]	Dose (ppm)	Final body weight (g)	Liver weight (g)	Liver : body weight ratio
1242	500	273 ± 20	12.98 ± 1.03	0.047 ± 0.002[c]
	50	297 ± 31	9.93 ± 1.40	0.033 ± 0.002
	5	295 ± 33	9.19 ± 1.16	0.031 ± 0.001
	0.5	290 ± 7	8.71 ± 0.67	0.030 ± 0.001
	0	287 ± 18	8.69 ± 0.69	0.030 ± 0.001
1248	500	307 ± 25	16.90 ± 2.31	0.055 ± 0.005[c]
	50	329 ± 16	11.59 ± 1.16	0.035 ± 0.003[d]
	5	290 ± 30	8.70 ± 1.64	0.030 ± 0.003
	0.5	274 ± 13	8.53 ± 0.95	0.031 ± 0.002
	0	318 ± 26	9.93 ± 1.24	0.031 ± 0.002
1254	500	240 ± 33	15.14 ± 2.53	0.063 ± 0.003[c]
	50	255 ± 24	9.43 ± 1.25	0.038 ± 0.003[c]
	5	259 ± 20	7.87 ± 0.68	0.030 ± 0.002
	0.5	262 ± 15	8.38 ± 1.23	0.032 ± 0.004
	0	272 ± 22	8.02 ± 0.89	0.029 ± 0.001
1260	500	267 ± 22	15.90 ± 2.56	0.060 ± 0.009[c]
	50	285 ± 15	11.96 ± 1.54	0.042 ± 0.004[c]
	5	278 ± 22	9.11 ± 0.85	0.033 ± 0.003
	0.5	259 ± 23	8.21 ± 0.65	0.031 ± 0.002
	0	286 ± 21	8.65 ± 0.98	0.030 ± 0.002

[a] Results are given as the mean of 6 rats ±SD.

[b] Polychlorinated biphenyl compounds containing 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.

[c] Significantly different from control group at $P < 0.001$ as judged by the student t test.

[d] $P < 0.05$.

III.10.2 Proliferation of smooth endoplasmic reticulum.

The initial increase in liver weight is due mainly to proliferation of smooth surfaced membranes of the endoplasmic reticulum, a network of interconnected channels present in the cytoplasm of most animal cells. The endoplasmic reticulum contains complexes of enzymes which play a role in metabolizing foreign substances such as drugs or environmental pollutants. These foreign substances are usually transformed into substances which can be more easily excreted by the body. A control step in metabolism of most foreign agents involves an oxidation reaction mediated by enzyme complexes (220). When liver samples are homogenized, the endoplasmic reticulum is broken up into particles referred to as microsomes and the enzymes are often referred to as microsomal enzymes. The liver microsomal enzymes responsible for metabolizing substances not normally expected to be present in the human body are sometimes referred to as drug-metabolizing enzymes. Norback and Allen (221) observed a proliferation of smooth endoplasmic reticulum (SER) in rats fed PCBs for one to five weeks. Similar proliferation of SER has been observed in mouse and monkey livers (197, 210).

The proliferation of SER is accompanied by increased activity of the microsomal enzymes responsible for metabolizing drugs and other foreign substances. Related observable changes in liver include increase in protein content and cytochrome P-450* content of microsomes.

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* Cytochrome P-450 -- a microsomal hemoprotein implicated as the terminal oxidase in hepatic enzyme systems.

The duration and intensity of action by many drugs, carcinogens and other environmental chemicals which enter the body depends to a large extent on the rates at which they are metabolized by the cytochrome P-450 containing mixed function oxidase system in liver cells (222). Alvares et al. (223) found that Aroclor 1254 treatment (25 mg/kg per day for 6 days) nearly tripled cytochrome P-450 concentrations in rat liver. Litterst et al. (219) observed a significant increase in microsomal cytochrome P-450 content in rats fed 50 and 500 ppm Aroclors 1242, 1248, 1254, and 1260 for four weeks. In addition, a P-450 increase was apparent at 5 ppm of Aroclors 1248, 1254 and 1260 (Table III.10.2). The increase in cytochrome P-450 content appears to be dose-related and to increase somewhat with increasing chlorine content.

III.10.3 Microsomal enzyme induction

Disruptions in normal enzyme activity have been observed even at dose levels which had no effect on liver weight or on liver-to-body weight/ratios (219). Several hepatic enzyme systems are affected at oral doses as low as 5 ppm (224) or even 0.5 ppm (219): "no effect levels" have not been found (Table III.10.3). A chronic exposure to low dose levels of PCB is likely to exact an effect on enzyme activity in human liver.

The ability of PCBs to induce enzymes was first shown by Risebrough et al. (225). Allen and Abrahamson (188) found that PCBs caused overall increase in enzyme activity of rat livers which varied from approximately 2 to 10 fold when expressed in relation to the

Table III.10.2 (from ref. 219)

Effect of PCB Treatment on Liver Microsomal Protein and Cytochrome P-450 Content of Rats[a]

Aroclor No.[b]	Dose (ppm)	Protein[c]	P-450[d]
1242	500	51 ± 2	1.26 ± 0.09[e]
	50	43 ± 4	0.69 ± 0.05
	5	45 ± 4	0.42 ± 0.03
	0.5	40 ± 3	0.51 ± 0.03
	0	45 ± 5	0.46 ± 0.04
1248	500	84 ± 2[e]	1.29 ± 0.11[e]
	50	54 ± 4	0.71 ± 0.04[e]
	5	54 ± 5	0.46 ± 0.02
	0.5	51 ± 4	0.59 ± 0.04
	0	66 ± 7	0.37 ± 0.05
1254	500	60 ± 3[e]	1.42 ± 0.12
	50	60 ± 4	0.88 ± 0.06
	5	45 ± 2	0.71 ± 0.04
	0.5	45 ± 2	0.61 ± 0.04
	0	40 ± 3	—
1260	500	100 ± 8[e]	1.24 ± 0.09[e]
	50	73 ± 4[e]	1.20 ± 0.10[e]
	5	60 ± 5	0.71 ± 0.04
	0.5	53 ± 3	0.54 ± 0.03
	0	47 ± 3	0.57 ± 0.02

[a] Values are the results ±SD of duplicate determinations conducted on a microsomal pellet from six pooled livers.

[b] PCB compounds containing from 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.

[c] Expressed as mg microsomal protein/g liver.

[d] Expressed as nmoles of cytochrome P-450/g of microsomal protein.

[e] Significantly different from control values at $P < 0.05$ as judged by the student *t* test.

Table III.10.3 (from ref. 219)

EFFECT OF PCB TREATMENT ON DEMETHYLATION ACTIVITIES IN HEPATIC MICROSOMES OF RATS[a]

		Substrate					
		Aminopyrine		Codeine		Ethylmorphine	
Aroclor No.[b]	Dose (ppm)	Units[c]	% of control	Units[c]	% of control	Units[c]	% of control
1242	500	14.2 ± 0.4^{d}	240	22.4 ± 0.3^{d}	245	15.9 ± 0.1^{d}	219
	50	9.6 ± 0.1^{d}	163	15.1 ± 0.4^{d}	165	11.2 ± 0.3^{d}	155
	5	7.6 ± 0.4	129	11.6 ± 0.2	127	8.7 ± 0.2	121
	0.5	9.3 ± 0.2^{e}	108	9.1 ± 0.2	99	7.8 ± 0.1	108
	0	5.9 ± 0.2	100	9.1 ± 0.1	100	7.2 ± 0.3	100
1248	500	17.4 ± 0.5^{d}	272	24.8 ± 0.4^{d}	258	17.6 ± 0.5^{d}	243
	50	10.4 ± 0.4^{d}	163	16.5 ± 0.4^{d}	171	12.1 ± 0.5^{d}	166
	5	7.6 ± 0.3	120	11.5 ± 0.3^{e}	119	9.6 ± 0.4^{d}	133
	0.5	5.1 ± 0.2^{e}	79	8.5 ± 0.3^{e}	88	7.4 ± 0.3	102
	0	6.4 ± 0.4	100	9.6 ± 0.1	100	7.2 ± 0.1	100
1254	500	19.3 ± 0.2^{d}	336	25.3 ± 0.3^{d}	264	17.9 ± 0.5^{d}	236
	50	16.6 ± 0.2^{d}	289	24.4 ± 0.5^{d}	253	17.9 ± 0.4^{d}	236
	5	8.9 ± 0.3^{d}	155	13.2 ± 0.3^{d}	138	11.1 ± 0.2^{d}	146
	0.5	7.2 ± 0.2^{d}	127	12.2 ± 0.2^{d}	127	9.6 ± 0.2^{e}	127
	0	5.8 ± 0.2	100	9.6 ± 0.2	100	7.6 ± 0.3	100
1260	500	22.2 ± 0.2^{d}	274	28.5 ± 0.5^{d}	197	17.4 ± 0.3^{d}	162
	50	18.6 ± 0.3^{d}	229	26.6 ± 0.3^{d}	183	18.0 ± 0.2^{d}	167
	5	13.0 ± 0.3^{d}	160	20.7 ± 0.3^{d}	143	15.9 ± 0.2^{d}	148
	0.5	9.6 ± 0.3^{d}	118	14.9 ± 0.3	103	12.4 ± 0.3^{e}	116
	0	8.1 ± 0.1	100	14.5 ± 0.1	100	10.7 ± 0.2	100

[a] Each value is the mean ±SD of 3 replicates.

[b] Polychlorinated biphenyl compounds containing 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.

[c] Expressed as μmoles of product formed/g liver/30 min.

[d] Significantly different from control group at $P < 0.01$ as judged by the student t test.

[e] $P < 0.05$.

total weight of the liver. Since PCBs are relatively resistant to metabolic breakdown, they persist as enzyme inducers. As the enzymes are continuously induced, hormones and perhaps other compounds may be metabolized at rates faster than normal over the whole life span of the organism. Increased metabolism of several hormones in birds and mammals has been demonstrated (225-227).

After a single oral dose of 50 mg/kg body weight Aroclor 1254 administered via stomach tube, microsomal enzyme activities increased until 24 hours after treatment and then either remained elevated or declined. Enzyme activities were still significantly increased over control values 48 hours after the single administration (228). Young rats are more susceptible to the enzyme inducing effects than adult rats (229).

With continuous exposure to high dietary levels of PCBs, a decrease is eventually observed in SER proliferation and concentric membrane arrays pervade the cytoplasmic reticulum. The induced levels of microsomal enzyme activity persist (221).

After termination of exposure, the enzyme inducing effect of PCBs appears to be reversible. Litterst and Van Loon (228) maintained rats on a diet of 50 ppm PCB for seven days. Discontinuation of PCB treatment resulted in a slow decay of the induced enzyme activity to approximately control levels after ten days.

Increased activities of the drug metabolizing enzymes aniline hydroxylase and aminopyrene n-demethylase were observed in pregnant rabbits exposed to single doses of 10 mg/kg Aroclor 1254 (217).

Carboxylesterase activity and the ability to degrade parathion to p-nitrophenol were significantly increased by the same doses. The effects of Aroclor 1221 were smaller than those of Aroclor 1254 at the same doses, but both Aroclors affected vitamin A storage after a single dose of 1 mg/kg (217).

Application of very small amounts of PCBs to the skin of experimental animals can cause a marked increase in mixed function oxidase activity (220).

A significant increase in activity of demethylating, reducing and hydroxylating microsomal enzymes was observed in livers of rats fed 150 μmoles per kg of food for 30 days. PCBs at this dose produced a significantly greater increase in activity than did DDT on all but the demethylating reaction where the effect was equal (228).

Specific enzyme activity, expressed per unit weight of microsomal protein or per unit weight of liver may increase or decrease under the influence of PCBs. However, when these data are expressed in relation to the total liver, a decided increase has been observed in activity of all enzymes assayed (188). PCB exposure can cause changes in activity of specific enzyme pathways in liver microsomes. The following alterations in enzyme activity have been observed:

- Nitroreductase -- Specific activity increased during the first week under the influence of Aroclors 1248 and 1254. Activity returned to near normal values during the second week and continued to decrease through the sixth week. In rats fed Aroclor 1262, a much greater increase in activity persisted (188).

  Litterst et al. (219) observed a striking increase in nitroreduction of p-nitrobenzoic acid. The effect was dose-related. The activity of nitroreductase in rats fed 500 ppm of Aroclor 1260 was 10 times the control values while all 4 Aroclors (1242, 1248, 1254, and 1260) at 0.5 ppm caused a 50 to 70 percent increase in activity (Figure III.10.1). The nitroreductase induction effect increased with increasing chlorine content (219). Nitroreductase activity levels continued to increase during four weeks of treatment with PCBs (228).

- Demethylase -- PCBs induced activity of demethylases in rat liver microsomes. The increase in amounts of product formed with increased PCB concentrations suggests that a dose-response relationship exists between any one specific Aroclor and this enzyme. Litterst et al. (219) observed that demethylase activity reached a peak at 54 percent chlorine content and then decreased in amplitude with Aroclor 1260 (Table III.10.3). Chen and DuBois (229), however, observed an increase in demethylase activity with a degree of chlorination for Aroclors 1221, 1254, and 1260.

  Even at the highest dose of all the Aroclors tested, demethylase activities do not exceed a level of 2 to 3 times the control values (219). In PCB-treated rats, demethylase activity reached the maximum level of induction within seven days (228).

  Vaino (230) observed a 16-fold increase in p-nitroanisole O-demethylase activity after treating rats for 6 days with daily injections of Clophen A50 (15 mg/kg).

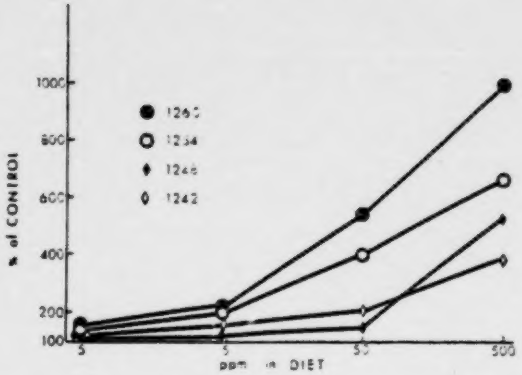
  Allen and Abrahamson (188) observed an increase in specific activity of rat liver demethylase throughout the 6-week PCB feeding period.

- Deethylase -- Wilson and Hansen (231) observed a marked increase in deethylation of p-nitrophenetole in microsomal preparations from sheep after feeding for 15 weeks with 20 ppm Aroclors 1242 or 1254 (Table III.10.4).

- Glucose-6-phosphatase -- Specific activity in rat liver decreased continuously for six weeks (188). Litterst et al. (219) also observed a decrease in specific activity of glucose-6-phosphatase in livers of PCB-dosed rats: the effect was most marked for Aroclor 1242 and was significant at the lowest dose tested (0.5 ppm for 4 weeks).

- Hydroxylase -- Although overall increase of hydroxylase activity has been consistently observed on administration of PCBs, patterns of change in specific hydroxylase activity observed by different investigators have varied.

Figure III.10.1 (from ref. 219)



Dose-response relationship of 4 Aroclors on nitroreduction of *p*-nitrobenzoic acid in rat liver microsomes. Each point represents the mean of 3 replicates. The SD values were all less than 5% of their respective means.

Table III.10.4 (from ref. 231)

Specific Activity of *p*-Nitrophenetole *O*-Deethylation Compared to the Slope of Lineweaver-Burke Kinetic Plots at Low (0.0125–0.15 mM) and High (0.15–2 mM) Substrate Concentrations

	Specific activity[a]			Slope	
Treatment	0.05 mM[b]	0.5 mM	1 mM	0.15–2 mM	0.0125–0.15 mM
Normal control	2.67	6.98	7.48	0.0233	0.0035
Normal 1242	4.07	6.66	7.61	0.0103	0.0022
Normal 1254	5.67	9.48	11.22	0.0074	0.0024
Low-protein control	5.44	8.40	10.36	0.0177	0.0025
Low-protein 1242	9.26	13.07	14.94	0.0094	0.0022
Low-protein 1254	13.72	16.82	18.35	0.0030	0.0023
High-fat control	10.70	16.94	17.31	0.0030	0.0023
High-fat 1242	4.55	7.36	7.30	0.0089	0.0023
High-fat 1254	8.42	12.60	14.08	0.0092	0.0034

[a] nmol/min/mg protein.

[b] Substrate concentration.

Vaino (230) observed a 7.5-fold increase in hepatic aryl hydrocarbon hydroxylase activity after treating rats for 6 days with consecutive daily injections of 15 mg/kg Clophen A50. A slight enhancement in the overall hydroxylation reaction was already observable 24 hours after a single intraperitoneal injection.

Allen and Abrahamson (188) observed a continuous decrease in specific activity of aromatic hydroxylase in rat liver for six weeks: this anomalous result is perhaps due to the very high dose used, which caused overt liver damage and ultimately death.

Litterst and Van Loon (228) observed two peaks in hydroxylase activity after PCB administration to rats. One peak occured 7 days after treatment and the other peak occured 28 days after treatment. Activity was slightly lower after 14 days than it had been after 7 days. Litterst and Van Loon used ^{14}C-pentobarbital as their substrate.

Alvares et al. observed an 11-fold increase in activity of benzo(a)-pyrene hydroxylase after application of microscope immersion oil containing PCBs to rat skin (222).

o Esterase -- Specific activity decreased continuously for rats on Aroclor 1248 and 1254 diets. With Aroclor 1262 there was a slight increase the first week, then specific activity leveled off (188).

By increasing activity of drug-metabolizing enzymes, PCBs can reduce the pharmacological effect of certain drugs. One parameter used to measure enzyme induction is reduction of hexobarbital or pentobarbital induced sleeping time (220). After treatment with PCBs, the rate at which rats metabolize the barbiturates to nonnarcotic substances is increased. Decrease in pentobarbital sleeping time following dietary administration of several PCB isomers was demonstrated in rats (232, 233), mice (234) and rabbits (235). Diminished pharmacological actions of zoxazolamine and hexobarbital have also been observed (236).

The various effects of PCBs on liver microsomal enzymes can be blocked by the prior administration of actinomycin D (5). Actinomycin D acts as an inhibitor of DNA-dependent RNA synthesis. Alvares _et al_. (223) interpreted the inhibitory effect of Actinomycin D on PCB-induced increases in enzyme activity as support of the view that PCBs enhance the synthesis of a distinct microsomal hemoprotein not present in liver of untreated rats.

There are substances other than PCBs which are also known to increase the activity of drug metabolizing enzymes in the liver microsomes. Litterst and Van Loon (228) compared the enzyme-inducing effect of Aroclor 1254 with the effects of DDT and phenobarbital. Rats were fed 5, 15, or 150 u moles per kg of food for 30 days. They concluded that at 150 u moles per kg food, PCBs were far more effective enzyme inducers than phenobarbital and at least as effective as DDT.

Two types of inducers of drug-metabolizing enzymes of the liver have been reported. One group, which includes phenobarbital, enhances the metabolism of a large variety of substrates. The other group, which includes such polycyclic aromatic hydrocarbons (PAH) as benzo(a)-pyrene and 3-methylcholanthrene, stimulates the metabolism of only a few compounds. The effects of these two groups of inducers on cytochrome P-450 differ. Cytochrome P-450 is a microsomal hemoprotein which has been implicated as the terminal oxidase in the hepatic enzyme systems. Treatment of rats with inducers from either group increases concentration of cytochrome P-450, but the PAH also induce formation of cytochrome P-448. Cytochrome P-448 differs from cytochrome P-450 in spectral properties and catalytic activities (223). Polychlorinated biphenyls appear to be a new type of hepatic enzyme inducer, sharing the properties of both groups. PCBs cause the formation of cytochrome P-448 (like the PAH group) while eliciting the more general enzyme induction response of the phenobarbital group.

Data of Vaino (230) support the hypothesis that PCBs form a new type of inducer group enhancing drug biotransformation in liver microsomes. PCBs were observed to produce a cytochrome P-448 with catalytic properties differing from those of P-448 induced by PAH (223, 230). PCBs were also able to increase the NADPH cytochrome reductase activity which is uninfluenced by PAH. Alvares _et_ _al_. (222) also reported induction of NADPH cytochrome c reductase in addition to cytochrome P-448 by PCBs.

Barbiturates and PAH both stimulate the UDP glucuronosyltransferase activity of liver microsomes. The enhancement of UDP glucuronosyltransferase activity caused by Clophen A50 was considerably greater than obtained by any of the previously known inducers (230).

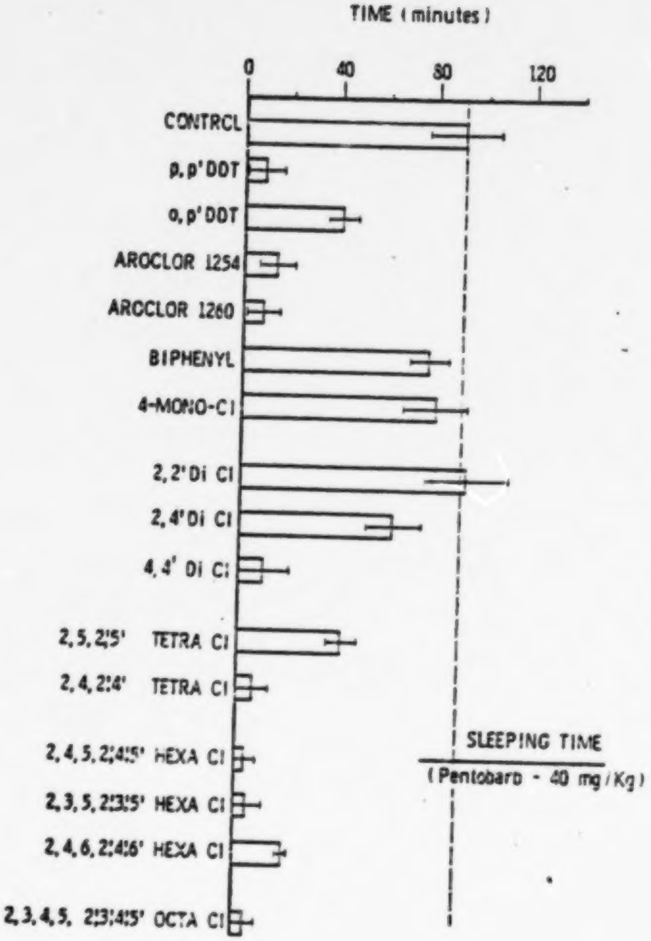
Wilson and Hanson (231) have also given evidence from the kinetics of O-deethylating reactions in sheep microsomal preparations that PCBs stimulate two distinct types of enzyme system.

Alvares and Siekevitz (237) have shown by means of gel electrophoresis that PCBs induce three distinct subunits of cytochrome P-450.

III.10.4. Structure-activity relationships.

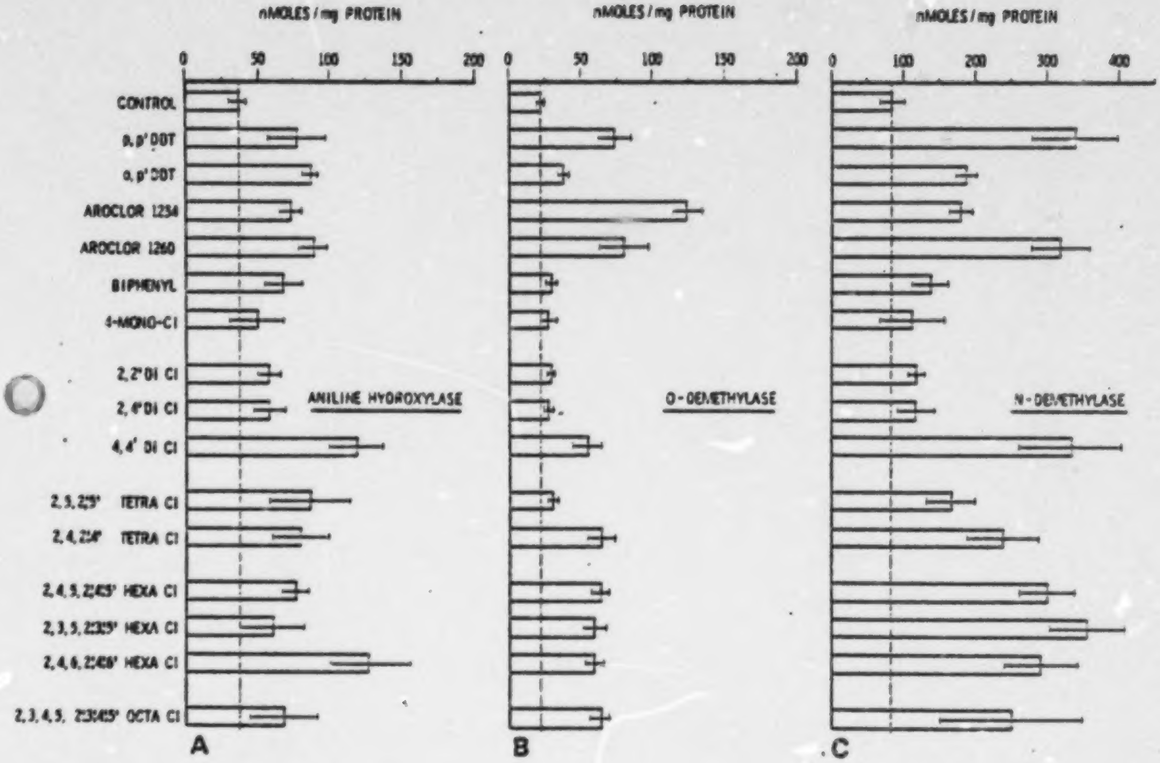
A number of recent studies have started to elucidate structure-activity relationships in enzyme induction by chlorobiphenyls (146, 224, 238-242). The results of some of these studies are summarized in Figures III.10.2 - III.10.6 (from ref. 242).

Figure III.10.3 summarizes the influence of the various agents tested on the hepatic mixed function oxidases aniline hydroxylase (A), p-nitroanisole O-demethylase (B), and aminopyrine N-demethylase (C). The most responsive enzymes were those closely associated with the smooth endoplasmic reticulum. The results *in vitro* reflected the observations of altered sleeping times *in vivo* (Figure III.10.2). One can see that, even with this limited number of pure chlorobiphenyls, not all caused effects of the same magnitude. To summarize the results of this study, treatment with biphenyl caused slight induction while 4-chlorobiphenyl did not. The mixed function oxidases were markedly induced by pure hexa- and octa-chlorobiphenyls and also by di- and tetra-chlorobiphenyls with chlorines substituted at the 4-positions of the rings. Considering the dichloro-isomers, when the 4- and 4'-positions were occupied, there was a much greater inductive effect for all of the enzyme activities than was observed with the 2,2'- and 2,4'-isomers. The same positional phenomenon was observed for the two tetra-chlorobiphenyls studied, the induction caused by 2,4,2',4'-tetra-chlorobiphenyl being much greater than that observed with the 2,5,2',5'-isomer. The results obtained for the higher chlorinated analogs suggested that the positions of the



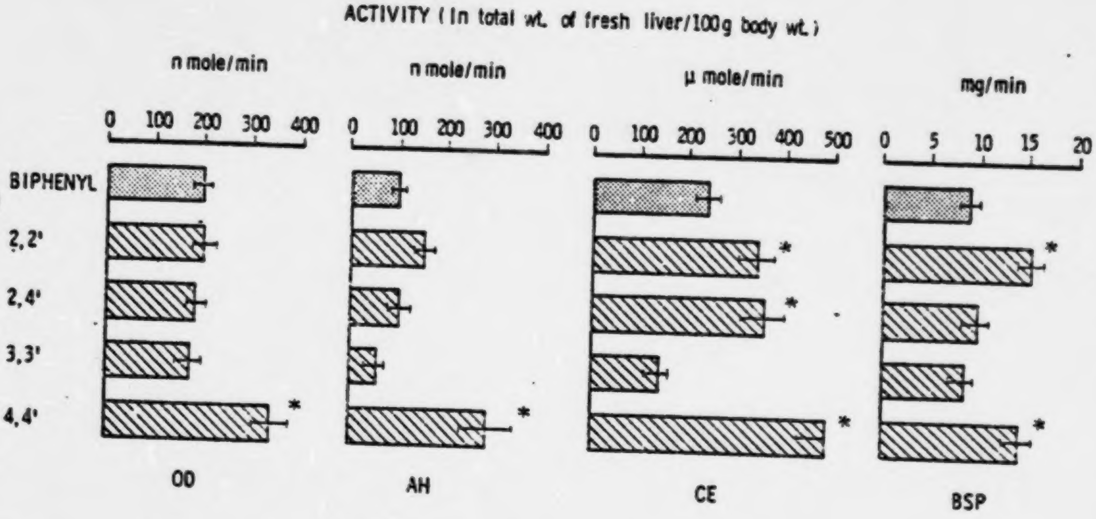
The effect of pretreatment of young male rats with DDT (o,p' and p,p'-isomers), Aroclor 1254 and 1260, biphenyl, and a series of isomerically pure chlorobiphenyls on the sleeping time produced by an injection of 40 mg/kg sodium pentobarbital.

Figure III.10.2 (from ref. 241)



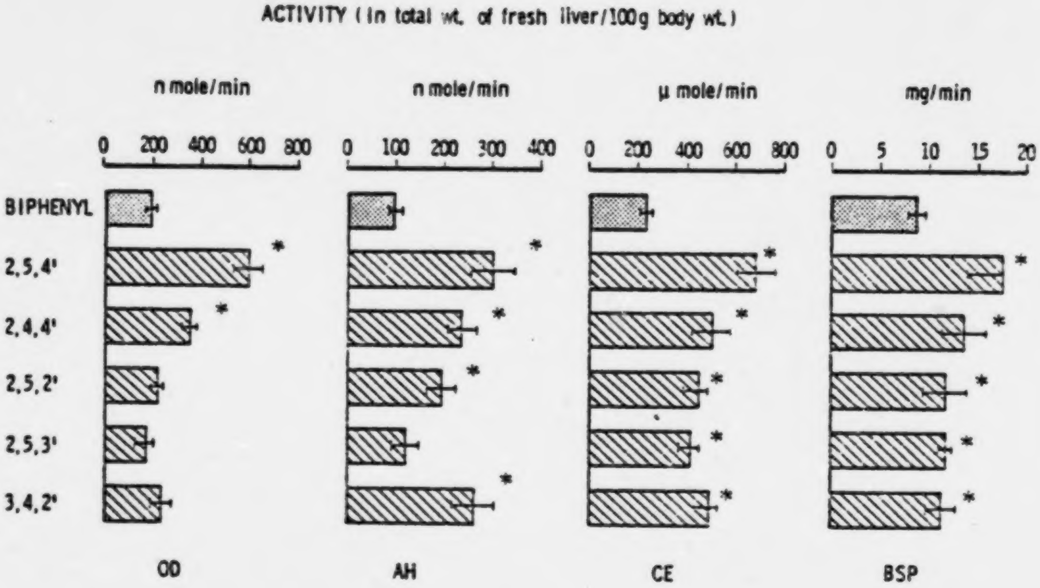
The effect of pretreatment of young male rats with DDT (o,p'- and p,p'-isomers), Aroclor 1254 and 1260, biphenyl, and a series of isomerically pure chlorobiphenyls on hepatic microsomal aniline hydroxylase (A), p-nitroanisole O-demethylase (B), and aminopyrine N-demethylase (C) activities. Activities are expressed as nmoles of product formed/mg microsomal protein/30min incubation.

Figure III.10.3 (from ref. 241)



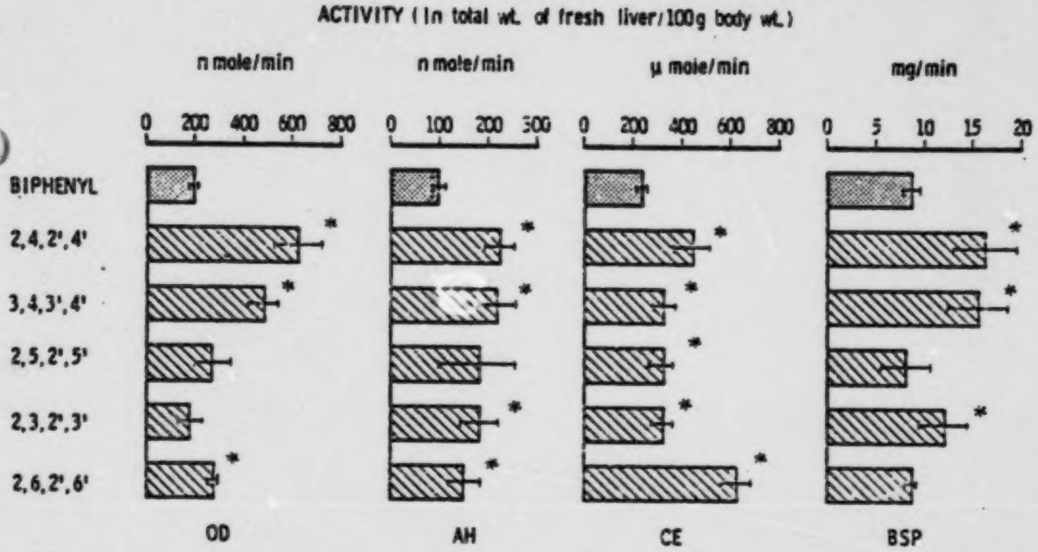
The effect of pretreatment of young male rates with biphenyl and a series of isomerically pure dichlorobiphenyls on hepatic p-nitroanisole O-demethylase (OD), aniline hydroxylase (AH), carboxylesterase (CE), and sulfobromophthalein-glutathione conjugating enzyme (BSP) activities. Animals were treated by i.p. injection for 3 consecutive days and were killed and assayed 96 hr after the last injection. The values (bars) represent the mean enzymatic activities ± S.D. (lines) of 18 control, biphenyl-treated rats and 6 animals per treated group. The asterisk (*) indicates values statistically different ($p < 0.55$) from biphenyl-treated controls.

Figure III.10.4 (from ref. 241)



The effects of pretreatment of young male rats with biphenyl and a series of isomerically pure trichlorobiphenyls on hepatic p-nitroanisole O-demethylase (OD), aniline hydroxylase (AH), carboxylesterase (CE), and sulfobromophthalein-glutathione conjugating enzyme (BSP) activities.

Figure III.10.5 (from ref. 241)



The effects of pretreatment of young male rats with biphenyl and a series of isomerically pure tetrachlorobiphenyls on hepatic p-nitroanisole O-demethylase (OD), aniline hydroxylase (AH), carboxylesterase (CE), and sulfobromophthalein-glutathione conjugating enzyme (BSP) activities.

Figure III.10.6 (from ref. 241)

chlorine atoms were not as important. These results, with the exception of the marked effects obtained following treatment with 4,4'-dichlorobiphenyl, confirmed the observations of other investigators who have found that penta-, hexa- and octa-chlorobiphenyls had greater enzyme-inducing potential than did low chlorine-containing biphenyls (205, 224, 239).

The influence of a series of di-, tri-, and tetrachlorobiphenyls on the selected enzymatic functions are shown in Figures III.10.4-6, respectively. With each series of isomers, a chlorine atom on the 4-position caused a more marked induction of hepatic drug-metabolizing enzyme activities than did a chlorine atom at any other position. As one increased the degree of chlorination, subsequent substitution at the 2-position was next in importance followed by substitution at the 3-position. The results conclusively demonstrated that not all isomers of the mono-, di-, tri-, and tetrachlorobiphenyls possess the same toxicologic properties, the position of chlorination being as important as degree of chlorination (242).

Fujita _et_ _al_. (239) also noted high specific activities of several isomers with 4,4'-substitution, including especially 3,4,3',4'-tetraCB, 2,4,5,3',4'-pentaCB, and 3,4,5,3',4',5'-hexaCB.

As a general summary of these results, for the mono-oxygenases closely associated with the hepatic endoplasmic reticulum, enhanced induction of activity was observed with highly chlorinated biphenyls and by low chlorine-containing congeners having chlorine atoms substituted at the 4- and 4'-positions irrespective of chlorination at other positions. For those enzymes less discretely localized in the hepatocyte, the position of the chlorine atoms appeared to be less important (242).

III.10.5. Enzyme induction by Aroclor 1016

Three studies have compared the activity of Aroclor 1016 in inducing liver enzymes with that of more highly chlorinated mixtures. Iverson et al. (243) compared the responses of rats after 21 daily oral doses of 1, 10 and 100 mg/kg of Aroclors 1016 and 1242. In male rats the responses were generally similar and if anything slightly larger for Aroclor 1016 (Table III.10.5): Aroclor 1016 caused significant increases in body weight gain, liver-to-body-weight ratio, and activity of aniline hydroxylase and aminopyrine demethylase at doses as low as 1 mg/kg, and increases in cytochrome P450 at 10 mg/kg. In female rats, by contrast, neither Aroclor caused significant changes at 1 mg/kg and the effects of Aroclor 1242 were more pronounced on all parameters measured except P450 at 10 and 100 mg/kg (Table III.10.6). Neither mixture had significant effects at 0.1 mg/kg (243).

Bickers et al. (244) compared the effects of Aroclors 1016 and 1254 on liver enzymes of male rats following six daily doses of 25 mg/kg. The effects of Aroclor 1254 were larger than those of Aroclor 1016 on each of the six enzyme functions measured (Table III.10.7) and Aroclor 1016 did not have significant effects on aniline hydroxylase or cytochrome b_5. The contrast between this result and the strong induction of aniline hydroxylase by Aroclor 1016 in

Table III.10.5 (from ref. 243)

Effect of Aroclor 1016 on male rats after daily oral dosing for 21 days

	DOSE 1016				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	74.0±4.6	90.7±2.8 **	83.1±4.1	90.4±4.4 **	122
LIVER % BODY WEIGHT	4.11±0.08	4.48±0.04 **	4.66±0.07 **	6.04±0.10 **	147
P_{450} nm/mg protein	0.92±0.04	0.90±0.05	1.43±0.11 **	1.76±0.17 **	181
ANILINE HYDROXYLASE μm/hr/g	2.09±0.10	2.45±0.13 **	4.50±0.35 **	5.60±0.28 **	269
AMINOPYRINE N-DEMETHYLASE μm/hr/g	1.04±0.11	1.44±0.10 **	2.39±0.19 **	2.56±0.20 **	246
LIVER PORPHYRINS μ[illegible]/g liver	0.18±0.01	0.20±0.03	0.16±0.02	0.44±0.04 **	244

** $P<0.05$

Effect of Aroclor 1242 on male rats after daily oral dosing for 21 days

	DOSE 1242				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	74.7±5.2	67.0±8.7	75.0±3.4 #	74.0±6.6	100
LIVER % BODY WEIGHT	3.97±0.06	4.26±0.06 **	4.64±0.11 **	6.83±0.18 **	172
P_{450} nm/mg protein	0.92±0.04	1.03±0.05 #	1.29±0.05 **	1.77±0.09 **	192
ANILINE HYDROXYLASE μm/hr/g	1.81±0.07	2.11±0.11 **	3.81±0.17 **	5.75±0.39 **	318
AMINOPYRINE N-DEMETHYLASE μm/hr/g	1.71±0.21	2.11±0.16	3.37±0.28 **	3.85±0.21 **	225
LIVER PORPHYRINS μm/g liver	0.21±0.03	0.19±0.02	0.30±0.05	1.07±0.11 **	510

** $P<0.05$.

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Table III.10.6 (from ref. 243)

Effect of Aroclor 1016 on female rats after daily oral dosing for 21 days

	DOSE 1016				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	29.2±2.7	30.5±1.8	35.5±2.4	33.6±1.9	115
LIVER % BODY WEIGHT	3.98±0.23	4.05±0.08	4.45±0.08	5.73±0.14 **	144
P_{450} nm/mg protein	0.62±0.08	0.77±0.05	0.78±0.04	1.10±0.03 **	177
ANILINE HYDROXYLASE µm/hr/g	1.10±0.06	1.13±0.09	1.23±0.13	2.48±0.19 **	226
AMINOPYRINE N-DEMETHYLASE µm/hr/g	0.40±0.02	0.44±0.02	0.38±0.04	0.70±0.05 **	175
LIVER PORPHYRINS µm/g liver	0.21±0.02	0.14±0.01	0.24±0.05	1.14±0.20 **	543

** $P<0.05$

Effect of Aroclor 1242 on female rats after daily oral dosing for 21 days

	DOSE 1242				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	28.7±2.3	28.0±3.7	35.3±3.7	18.3±5.5 **	64
LIVER % BODY WEIGHT	3.63±0.13	3.71±0.11	4.19±0.09 **	6.18±0.22 **	170
P_{450} nm/mg protein	0.85±0.05	0.82±0.03	1.14±0.04 **	1.50±0.05 **	177
ANILINE HYDROXYLASE µm/hr/g	1.01±0.02	1.08±0.03	1.84±0.10 **	3.01±0.23 **	298
AMINOPYRINE N-DEMETHYLASE µm/hr/g	0.45±0.01	0.48±0.05	0.81±0.02 **	1.12±0.12 **	249
LIVER PORPHYRINS µm/g liver	0.20±0.04	0.23±0.02	0.44±0.06 **	1.76±0.30 **	871

** $P<0.05$

Table III.10.7 (from ref. 244)

Changes in microsomal hemoprotein levels and N-demethylase and hydroxylase activities in response to Aroclor 1254 and Aroclor 1016[1]

Measurement	Controls	Aroclor 1016	Aroclor 1254
Mg microsomal protein/ g liver, wet weight	25.14 ±0.85	27.07 ±0.58	28.88 [2] ±0.88
Aniline hydroxylase, mμmoles p-aminophenol/mg protein/hr	36.42 ±1.55	35.83 ±1.90	48.06 [2] ±2.82
Ethylmorphine N-demethylase, umoles HCHO/mg protein/hr	0.349 ±0.022	0.498 [2] ±0.036	1.075 [2] ±0.056
Cytochrome P-450, mμmoles /mg protein	0.637 ±0.027	0.884 [2] ±0.051	1.776 [2] ±0.109
Cytochrome b_5, mμmoles /mg protein	0.399 ±0.019	0.423 ±0.009	0.561 [2] ±0.014
Zoxazolamine paralysis time, minutes	549 ±58	275 [2] ±13	0 [2,3]
Hexobarbital sleeping time, minutes	101 ±5	48 [2] ±4	24 [2] ±1

[1] Rats were pretreated with Aroclors 1016 or 1254, dissolved in corn oil, at a dosage of 25 mg/kg/day for 6 days. Controls received corn oil only. Each value represents mean ± S.E. for 5 rats.

[2] Values significantly different from the respective control values ($P<0.05$).

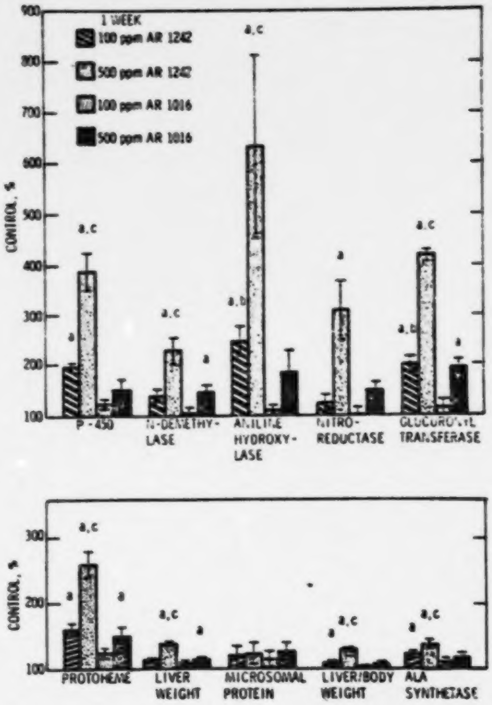
[3] No loss of righting reflex occurred in any of the 5 rats.

the experiment of Iverson _et al_. (243, Table III.10.5) probably results from the more extended dosage regime in the latter experiment, and emphasizes the complexity of the effects under review.

Goldstein _et al_. (245) compared the responses of female rats to Aroclors 1016 and 1242 after feeding at 100 and 500 ppm. After one week of feeding there were marked differences in response: Aroclor 1242 had markedly increased liver weight and all drug metabolizing systems tested including cytochrome P-450, N-demethylase, nitroreductase, aniline hydroxylase, and glucuronyl transferase, whereas Aroclor 1016 had produced only minimal effects (Figure III.10.7). After six months' exposure, however, the effects of the two mixtures were much more similar (Figure III.10.8). Thus the action of Aroclor 1016 on female rats appears to involve a time lag. It should be noted also that Goldstein _et al_. tested only female rats, whereas Iverson _et al_. (243) found that males were more sensitive to Aroclor 1016. Although in some experiments Aroclor 1016 had less pronounced effects on hepatic enzyme systems than Aroclor 1242, its effects thus appear similar after prolonged exposure.

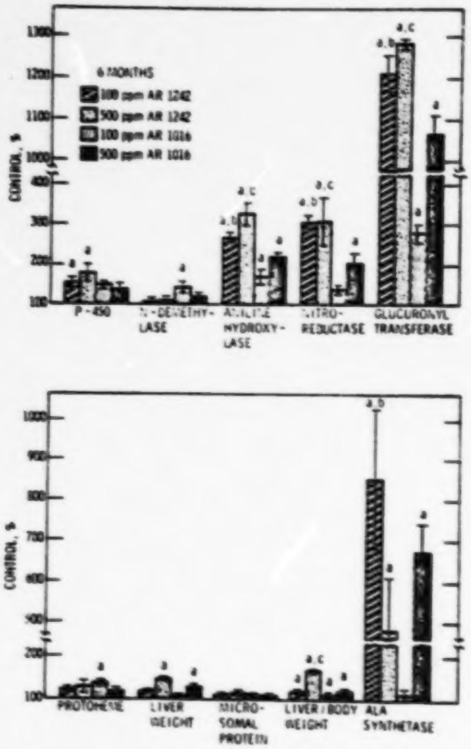
III.10.6. Enzyme induction by PCDFs.

No studies appear to have been reported of the activity of PCDFs in inducing hepatic microsomal enzymes in mammals. In chickens, 2,3,7,8-TCDF is as potent as 2,3,7,8-TCDD in inducing aryl hydrocarbon hydroxylases in the embryo (36), but it is much less active in inducing cytochrome P-450 in the hatched chick (146, 147).



. Effect of Aroclors 1016 and 1242 on cytochrome P-450, drug-metabolizing enzymes, protoheme, liver weight, liver microsomal protein, and ALA synthetase activity at 1 wk. Groups of six female rats were fed 100 or 500 ppm of Aroclors 1016 or 1242 and sacrificed at 1 wk. Values represent means ± SE. a—Significantly greater than controls at $p < 0.05$. b—100 ppm Aroclor 1242 significantly greater than 100 ppm Aroclor 1016, $p < 0.05$. c—500 ppm Aroclor 1242 significantly greater than 500 ppm Aroclor 1016, $p < 0.05$.

Figure III.10.7 (from ref. 245)



Effect of Aroclors 1016 and 1242 on cytochrome P-450, drug-metabolizing enzymes, protoheme, liver weight, liver microsomal protein, and ALA synthetase activity at 6 mo. Groups of six female rats fed 100 or 500 ppm of Aroclors 1016 and 1242 were sacrificed at 6 mo. Values represent means ± SE. a—Significantly greater than controls at $p < 0.05$. b—100 ppm Aroclor 1242 significantly greater than 100 ppm Aroclor 1016, $p < 0.05$. c—500 ppm Aroclor 1242 significantly greater than 500 ppm Aroclor 1016, $p < 0.05$.

Figure III.10.8 (from ref. 245)

III.10.7. Synergistic effects on enzyme induction.

Araki and Tanaka (246) reported significant synergistic effects between PCBs, DDT and BHC (HCH) in inducing hepatic microsomal enzymes *in vivo*. Using hexobarbital sleeping time as the indicator of enzyme activity, they found that the activities of DDT and β-HCH were about one-eighth of those of the same dose of PCBs (KC-400). When a small dose of DDT or β-HCH was combined with a very small dose of PCBs (each about one-quarter of the minimal effective dose when tested singly) the inducing activity was strongly potentiated.

III.10.8. Structural changes in liver.

Structural changes in liver cells, in addition to proliferated smooth endoplasmic reticulum, include: increase in lipid droplets within the cytoplasm, the formation of numerous multi-layered concentric membrane arrays, increase in numbers of microbodies and lysosomes within cells, and reduction of rough endoplasmic reticulum.

Microscopically, enlargement of individual hepatocytes was apparent. Present in the abundant cytoplasm of these cells were numerous small fat vacuoles, proliferated endoplasmic reticulum and large concentric membrane arrays (CMA). The CMAs encircled lipid droplets and other cytoplasmic organelles. There was a moderate increase in lysosomes. Other organelles, including the nucleus, were normal in appearance (188).

The CMAs may represent a means by which the hepatic cells are able to incorporate more membranes in a given area, thereby enhancing metabolic activity of the cell. CMAs were observed in rats after three weeks on a PCB-containing diet. The arrays increased in size and complexity in direct relationship to the time the rats stayed on the experimental diet (188).

Several other liver lesions have been observed after exposure to PCBs. Dietary exposure (500 ppm) of Sherman rats to Aroclor 1254 for 6 to 8 months produced extensive areas of adenofibrosis in the liver. Fatty metamorphosis, accumulation of brown pigment in hepatic macrophages and Kupffer cells are also seen (187, 189, 208; Tables III.9.4, III.9.5). Adenofibrosis is firm and grayish white, with a pitted or granular surface. On microscopic examination it consists of fibrosis and proliferated, atypical glandular epithelium that forms ducts and cysts. The cysts usually contain mucus or cellular debris (189). Some clusters of atypical glandular cells resemble pancreatic tissue in appearance and staining properties (247). The relation of these and other liver lesions to the development of neoplasia is discussed below under Carcinogenesis (Section III.14).

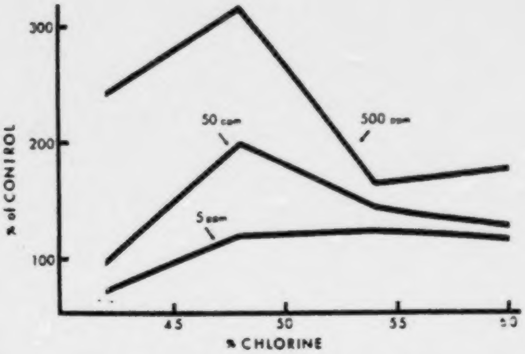
Nishizumi reported some variation in size of nucleus of mouse liver cells after 17 weeks of oral administration of PCBs (1.5 mg/day). He also noted enlargement of Kupffer's cells in livers of PCB-treated mice and monkeys (210).

III.10.9. Changes in biochemical composition of the liver.

Biochemical data obtained from liver homogenates of PCB-treated rats clearly indicates hypertrophy of the liver. As a result of membrane proliferation and more numerous ribosomes, there was an increase in protein content and RNA levels. Increased protein content of liver homogenate indicates more protein containing membranes within the cells. More ribosomes and the related increase in RNA would be necessary for production and maintenance of the elaborated membrane systems. These ribosomes are apparently not attached to the endoplasmic reticulum. A relative decrease in DNA, when expressed in relation to total liver weight, further signifies the proliferated state of the cytoplasmic components. There was no appreciable change in total DNA of liver or in amount of DNA per cell (188).

Changes in biochemical composition of the liver after exposure to PCBs include a significant increase in total lipid content (182). Triglyceride content of the liver increased with increased dosage of PCBs (from 5 to 500 ppm) in the diet (219). This increase was greater with Aroclor 1248 than with 1242, 1254 or 1260 (Figure III.10.9). The ratio of phospholipids to protein increased and the ratio of cholesterol to protein increased (221). The increase in liver lipid following PCB exposure is due in part to an increase in lipid synthesis and in part to a decrease in transport from the liver (248). Marked increases in liver lipids have also been noted in treated rabbits (256).

Allen and Abrahamson (188) observed compositional changes in liver microsomes of PCB-treated rats. Increase in protein content of microsomes was apparent soon after ingestion of PCBs and persisted throughout the four-week examination period. The levels of



Effect of dose and chlorine content of Aroclors on hepatic triglyceride concentrations in rats after 4 wk. Each point represents the mean of 3 replicates. With one exception all SD values were less than 10% of their respective means (SD of the 5 ppm dose of Aroclor 1248 was 18% of its mean).

Figure III.10.9 (from ref. 219)

phospholipids also increased moderately. There was a decided decrease in cholesterol and RNA in all experimental liver microsomes.

The increase in microsomal protein further substantiates the proliferated state of the endoplasmic reticulum since there would be more membranes per gram of liver. The decrease in microsomal cholesterol and the moderate increase in phospholipid content were, however, unexpected since proliferation of endoplasmic reticulum is usually accompanied by increases in protein, phospholipid and cholesterol at approximately the same rate. The decrease in microsomes is compatible with a proliferated state of the smooth endoplasmic reticulum. The increase in microsomal protein is related to an enhanced activity of the microsomal enzymes (188).

Bitman et al. (166) observed a very striking decrease of about 50% in liver concentration and content of vitamin A in rats fed 100 ppm Aroclor 1242 for 2 months. A decrease in liver storage of vitamin A in rats is also a symptom of repeated exposure to other chlorinated hydrocarbons (166, 5, 174).

III.11. Induction of Porphyria.

Porphyria cutanea tarda in humans is an acquired defect in hepatic porphyrin metabolism, characterized by uroporphorinuria (excretion of porphyrins in the urine), photosensitivity as manifested by blisters, and mechanical fragility of the skin (5). The hepatic porphyria which is responsible for the increase in porphyrins and for the skin photosensitivity can be produced experimentally by a number of drugs and chemicals which have the ability to stimulate activity of the initial enzyme in the heme synthesis, δ-aminolevulinic acid (ALA)-synthetase (249: see Figure III.11.1).

PCBs produce hepatic porphyria in animals. Goldstein et al. (250) observed hepatic porphyria in Sherman rats exposed to Aroclor 1254 in the diet for at least seven months. The porphyria resembled human porphyria cutanea tarda. When female rats were fed 100 ppm Aroclor 1254 in the diet, they developed hepatic porphyria in two to four months. At this time, the urinary porphyrin excretion increased rapidly. Uroporphyin excretion was elevated to a maximum of 540-fold, coproporphyrin, porphobilinogen 50-fold and ALA 18-fold. Massive amounts of 8- and 7-carboxyporphyrins accumulated in the liver. The major porphyrins excreted in the urine of PCB-treated rats were 8-carboxyporphyrin (73%) and 7-carboxyporphyrin (16%). Substantial increase in amounts of 5- and 6-carboxylic porphyrins were also observed. Excretion of coproporphyrin increased 10-fold while excretion of uroporphyrin increased 1400-fold. In control rat urines, coproporphyrin made up 81% of the porphyrin fraction. Porphyrin concentrations were high in livers of PCB-dosed rats but only a trace of porphyrin was found in liver samples in control rats.

The tremendous accumulation of uroporphyrins in the liver and urine of rats fed Aroclor 1254 suggests that PCBs may affect uroporphyrin formation or utilization. Hemosiderin has been found in the liver of rats treated with Aroclor 1254 (187). A partial block in heme synthesis may be involved in PCB-induced porphyria.

Induction of porphyria by PCBs is dependent on protein synthesis, endogenous iron, and perhaps cytochrome P-450 (251). There

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The porphyrin haem biosynthetic pathway.

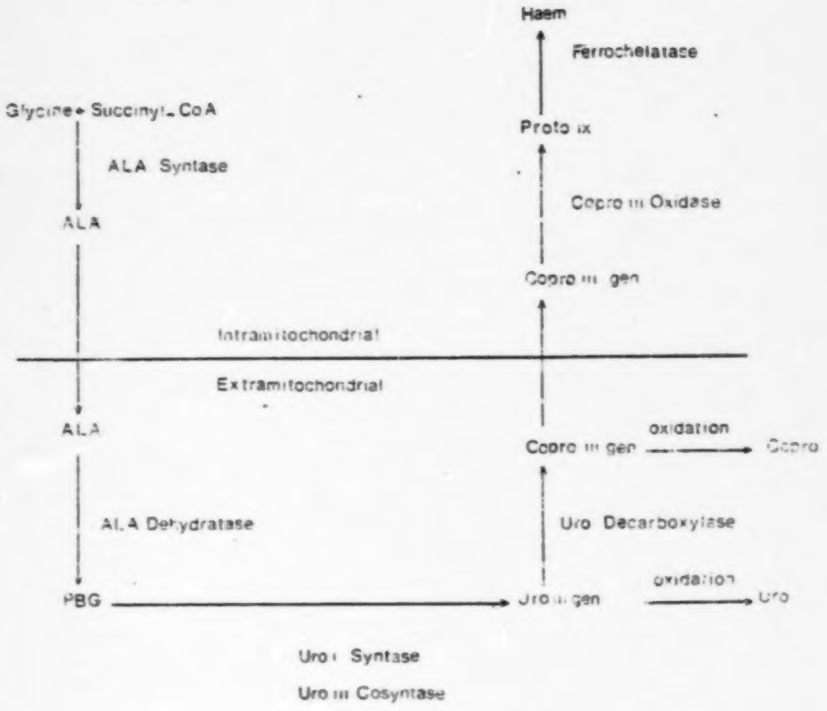
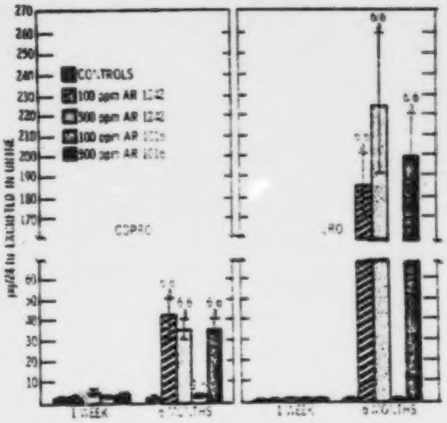


Figure III.11.1 (from ref. 248)

is some evidence that the active agent is an unstable metabolite, perhaps a hydroxylated chlorobiphenyl (251).

Aroclors 1016 and 1242 also induce porphyria in rats (243, 245). After dosing at 10 and 100 mg/kg/day for 21 days, both mixtures caused a significant accumulation of porphyrins in both sexes, but Aroclor 1242 was nearly twice as effective at 100 mg/kg and produced a significant effect at the lower dose (Tables III.10.5, III. 10.6). After feeding at 100 and 500 ppm, both Aroclors induced porphyrins in liver and urine (Figure III.11.2, Table III.11.1), but Aroclor 1016 did so only at the higher dose. Induction of ALA-synthetase followed the same pattern (Figure III.10.8).

Rabbits developed symptoms of porphyria after application of Clophen A60, Phenoclor DP6 and Aroclor 1260 to their back skin (204). Increased fecal excretion of coproporphyrin and protoporphyrin was observed in rabbits with a chromatographic fraction of the PCB mixtures which would contain any dibenzofurans present. Hence Vos suggested that porphyria is caused by PCBs themselves and not by PCDFs (128, 204). This has been supported by the recent work of Goldstein _et_ _al_. (146, 193), who found that mice fed 3,4,5,3',4',5'-hexaCB developed porphyria, but those dosed with 2,3,7,8-TCDF did not. In the same experiment 2,3,7,8-TCDD induced porphyria in mice (146), but TCDD is not a potent inducer of ALA-synthetase in other species of mammals (202, 252).



Effects of Aroclors 1016 and 1242 on urinary coproporphyrin and uroporphyrin excretion. Treatment is described in Fig. 3. Values represent means ± SE. The numbers above the bars represent the proportion of animals whose individual values were outside the 99% confidence limits of the controls at *p* < 0.01. a—Significantly different from controls, *p* < 0.01.

Figure III.11.2 (from ref. 245)

Table III.11.1 (from ref. 245)

EFFECT OF AROCLORS 1016 AND 1242 ON LIVER PORPHYRINS IN THE RAT[a]

Treatment	1 Week 7-COOH	1 Week 8-COOH	6 Months 7-COOH	6 Months 8-COOH
Control	Trace[b]	Trace[b]	Trace[b]	Trace[b]
100 ppm Aroclor 1242	Trace[b]	Trace[b]	164 ± 27	534 ± 61
500 ppm Aroclor 1242	Trace[b]	Trace[b]	227 ± 59	669 ± 146
100 ppm Aroclor 1016	Trace[b]	Trace[b]	Trace[b]	Trace[b]
500 ppm Aroclor 1016	Trace[b]	Trace[b]	89 ± 23	343 ± 65

[a] Porphyrins were methylated and analyzed by thin-layer chromatography. Female rats were treated as described in Fig. 1. Results are given as the mean ± SE.

[b] <1 μg/g.

III.12. Miscellaneous Biochemical Effects

III.12.1. Hypertriglyceridemia

In addition to the increases in liver lipids noted above in Section III.10.8, PCBs have been shown to cause increases in serum lipids in rats (181, 253) and in rabbits (254, 255). The doses used to show these effects were quite high (100-300 mg/kg/day in rats, 10-100 mg/kg/day in rabbits).

III.12.2. Effects on the adrenal gland

Wassermann *et al*. (257) observed a rise in the activity of the zona fasciculata of the adrenal gland in PCB-treated rats. After ten weeks of receiving 250 ppm of Aroclor 1221 in their drinking water, rats had a mean corticosterone plasma level twice as high as control rats. Morphological changes in the zona fasciculata of the adrenal gland also indicated increased activity, corroborating the biochemical data.

III.12.3. Effects on thyroxine.

Hepatic conjugation of thyroxine to glucuronic acid and excretion in the bile of thyroxime-glucuronide is a major route of thyroxine metabolism. Thyroxine-glucuronide formation is rate limiting for biliary thyroxine excretion.

The effects of PCB on thyroxine metabolism resemble the effects of such polycyclic aromatic hydrocarbon (PAH) enzyme inducers as 3, 4-benzo(a)pyrene and 3-methylcholanthrene. The polycyclic hydrocarbon type of hepatic microsomal enzyme inducer enhances biliary thyroxine-glucuronide formation (258). After daily treatment of rats with 25 mg/kg body weight of Aroclor 1254 for four days, biliary thyroxine excretion increased 4- to 5-fold. The ratio of iodine in bile to iodine in plasma was elevated tremendously. The rate of clearance of thyroxine from the blood through biliary excretion was greatly increased by PCB treatment. In PCB-treated animals, the proportion of free thyroxine in bile was reduced while the proportion of thyroxine in its metabolized form (thyroxine-glucuronide) was increased.

In PCB-treated rats, serum protein-bound iodine concentrations were less than half of normal. This may be attributed to enhanced biliary excretion of thyroxine and also to inhibition of thyroid hormone binding to plasma proteins. PCB, administered *in vivo* or added *in vitro* to rat serum, reduced binding of thyroid hormone to serum protein. This inhibition of plasma protein-binding of thyroxine may, in turn, contribute to the enhancement of biliary thyroxine excretion induced by PCB (258).

PCBs increased thyroid function, as shown by elevated uptake (258). For a review of effects of PCBs on the thyroid, see Jefferies (174).

III.12.4. Hematological effects.

Changes in blood composition and bone marrow histology have been observed in animals after exposure to PCBs.

Allen *et al*. (195) found that hematological changes developed gradually in rhesus monkeys fed diets containing 300 ppm Aroclor 1248.

Over three months, there was a decrease in hemoglobin of approximately 2 g/100 ml and a decrease in hematocrit from 40 to 33%. No major modifications of total white cell count were noted. However, there was a gradual decrease in the number of lymphocytes and a concomitant increase in neutrophils. There was also a decrease in total serum protein of 1.5 - 2 g/100 ml. A gradual shift in albumin/globulin ratio of serum protein also occurred. Allen et al. (215) speculate that PCBs may have an inhibiting effect on the red-blood-cell-forming tissue of bone marrow.

Bruckner et al. (259) found some evidence of anemia in rats given intraperitoneal injections of Aroclor 1242. Hematocrit, erythrocyte count, erythrocyte diameter, mean corpuscular hemoglobin concentration, and total blood hemoglobin concentration were all somewhat lower in PCB-dosed animals than in controls. An elevation in the leukocyte count was noted in the blood of PCB-dosed rats, with a large increase in the proportion of circulating neutrophils. Increased leukocyte counts were a consistent finding also in dogs chronically exposed to PCBs (212).

Other changes in blood of PCB-dosed animals observed by Bruckner et al. (259) include an elevation of serum iron and a decrease in both plasma corticosteroid and glucose concentration. Blood urea nitrogen concentration and serum bilirubin values were unchanged. The elevated serum iron and the decrease in hemoglobin concentration suggest a decreased incorporation of iron into protoporphyrin. Bruckner et al. (259) decided that the mild reduction in erythrocyte count in PCB-dosed animals was not due to hemolytic action since they found identical bilirubin values in serum of PCB-dosed and control rats. No alteration in osmotic fragility of erythrocytes was observed.

III.12.5. Enzyme inhibition.

PCBs have an inhibitory effect on some enzymes. Hendrickson and Bowden (260) demonstrated *in vitro* competitive inhibition by PCBs of lactic dehydrogenase in rabbit muscle. Lactic dehydrogenase is the terminal enzyme of anaerobic glycolysis.

Sharp *et al*. (261) showed that beef brain and rabbit kidney $(Na^+ + K^+)$ - ATPases were inhibited by PCBs. The $(Na^+ + K^+)$ATPase is generally thought to comprise the membranal sodium pumping system. The extent of enzyme inactivation increased as the degree of chlorination increased (262). Sharp *et al*. (261) obtained experimental evidence to support the hypothesis that PCBs inactivate membrane $(Na^+ + K^+)$ATPases by interfering with the stabilizing function of acidic phospholipids. Similar effects have been reported on ATPases in fish and have been linked with disruption of osmoregulation (127).

III.13. Immunosuppressive Effects

The ability of PCBs to inhibit immune responses has been demonstrated in several mammalian species. Following dermal exposure of rabbits to PCBs, Vos and Beems (204) observed atrophy of the thymus and a reduction in the number of germinal centers in spleen and lymph nodes. The thymus, spleen and lymph nodes are all lymphatic structures vital to the body's immune defense system. The germinal centers in spleen and lymph nodes are the principal productive field of lymphocytes. The thymus secretes a hormone that enables lymphocytes to develop into plasma cells which can synthesize antibodies against foreign proteins. Vos and Beems (204) also observed a reduction in total white cell counts in rabbits whose skin had been painted with PCBs. These observations suggest immunosuppressive action of PCBs.

Vos and de Roij (263, 129) reported a function test of the immunological system on three groups of twelve female albino guinea pigs fed 0, 10 and 50 ppm Aroclor 1260 for eight weeks. Tetanus toxoid injection was used to test the humoral immune response. They observed a significantly reduced number of γ-globulin-containing cells in lymph nodes of PCB-fed guinea pigs stimulated with tetanus toxoid, together with a significantly decreased γ-globulin level in the serum of toxoid-stimulated animals fed 10 ppm PCB. These observations demonstrated that some immunosuppression is produced by PCB-feeding.

Vos and van Driel-Grootenhuis (264) investigated cell-mediated immunity as well as the humoral immune response in guinea pigs. A suppression of the humoral immunity, after stimulation with one dose of tetanus toxoid was found at the 50 ppm level. The antitoxin levels were significantly decreased. The number of antitoxin-producing cells in skin reactions after tuberculination was the parameter of cell-mediated immunity. This test indicated a significant reduction in cell-mediated immunity at the 50 ppm level of PCBs in the diet.

Major effects on the lymphoid system due to the suppressive activity of PCBs included strongly reduced weights of the thymus, lymph node and, to a lesser extent, of the spleen (128, 264). Atrophy of the thymus in newborn rodents reduced the capacity of these animals to produce serum antibodies, resulting in wasting disease (cachexia). Such animals are then incapable of rejecting foreign skin grafts (5). Cachexia and depletion of the lymphoid system were observed in PCB-fed guinea pigs (264).

Koller and Thigpen (265) compared effects of Aroclors 1221, 1242 and 1254 on the humoral antibody response of rabbits exposed to pseudo-rabies virus. Rabbits fed all three PCB formulations had significantly lower serum-neutralizing antibody titers to pseudorabies virus than did rabbits who were not given PCB formulations. The serum titers from rabbits given PCBs were approximately one-half those of controls. Aroclor 1242 produced the lowest titers, succeeded by Aroclor 1254 and then Aroclor 1221.

Atrophy of the thymus and/or the spleen has been reported in several other species treated with PCBs, including chickens (146, 147, 70), mice (146, 147), monkeys (197) and pigs (216). Atrophy of the thymus and spleen are also striking effects of TCDF in chickens, guinea pigs and mice (70, 71, 146).

Immunosuppressive effects of PCBs may have been involved in several instances in which PCB-treated animals appeared more susceptible to disease than controls. These cases included increased susceptibility of piglets to septicemia (216), of ducks to viral hepatitis (178) and of fish to fungal disease (111).

Another potentially serious consequence of immunosuppression is an increased susceptibility to carcinogens, since suppression of immune response may make an animal more susceptible to establishment of neoplastic cell lines and development of malignant tumors.

III.14. Carcinogenic and Co-Carcinogenic Effects

Studies of carcinogenic and co-carcinogenic effects of PCBs are reviewed in detail in Appendix D and only a brief summary is included here.

In a long-term study by Kimbrough et al. (266), Sherman rats fed 100 ppm Aroclor 1260 in the diet for 21 months developed hepatocellular carcinomas (26/184) and neoplastic nodules (144/184) in the liver, whereas 173 control animals developed only one carcinoma and no neoplastic nodules. The terminology used to describe the liver lesions is that developed at a recent workshop of experts in liver pathology (269), where some of the slides from the PCB-treated animals were reviewed. The study reported in ref. 266 followed a sequence of shorter-term studies in which the earlier stages of development of liver lesions in PCB-treated rats had been described (see Section III. 10.3); in one of these studies, liver nodules had been reported in the second generation of rats exposed to 20 ppm Aroclor 1254 at an age of only 330 days (179). Other significant findings in the livers of treated rats were adenofibrosis (cholangiofibrosis) (189) and abnormal pancreatic-type tissues (247). The significance of these findings is not clear. Adenofibrosis has been reported in the livers of rats fed with carcinogens (268); some experts believe that they have the potential to develop into cholangiocarcinomas (283), although an earlier review concluded that they were not convincingly pre-cancerous (284).

In another long-term study (207), groups of Charles River rats were fed with 1, 10, and 100 ppm Aroclor 1242, 1254, and 1260 for 24 months. Although the initial report indicated only mild liver lesions (207), a more recent review (68) reports the occurrence of lesions classified as nodular hyperplasia, hepatomas and cholangiohepatomas in 44/74 of the rats surviving near to the end of the experiment in the groups fed 100 ppm of all three Aroclors. Although there are some terminological problems and findings have not been adequately reported, these results appear consistent with those of Kimbrough *et al*. and suggest an oncogenic potential for all three Aroclors. There was also an excess incidence of pituitary tumors (chromophobe adenomas) in all treated groups in this experiment.

Other studies reviewed in Appendix D were conducted for much shorter periods and generally at higher dose levels; they constitute little more than preliminary screening tests. Kanechlors KC-500, KC-400, KC-300 induced nodular hyperplasia and cholangiofibrosis, but not hepatocellular carcinomas, in rats within 28-52 weeks (190). KC-400 induced multiple adenomatous nodules in the livers of rats within 400 days (271). Aroclor 1254 induced adenofibrosis and hepatomas in mice fed for only 6-11 months (191). KC-500 induced nodular hyperplasia and hepatocellular carcinoma in mice within 32 weeks, whereas KC-400 and KC-300 did not do so within the same period (192).

These six studies are consistent in showing that PCB mixtures with 42-60% chlorine content induce neoplastic and pre-neoplastic lesions in the livers of rats and mice, and that this induction is very rapid at high dietary levels (250-500 ppm). Because most of the studies have been terminated early, the full development of the lesions has not yet been studied. No long-term feeding studies have yet been reported which have failed to show some evidence of neoplastic change: the longest such study was terminated after 12 months (69).

Among co-carcinogenic studies conducted in vivo, KC-500 markedly promoted or accelerated the hepatocarcinogenicity of α-BHC and β-BHC in mice (192). However, KC-400 did not promote the development of cervical carcinomas in mice induced by methylcholanthrene (272), and KC-500 inhibited or delayed the hepatocarcinogenicity of three other carcinogens in rats (273).

Numerous studies have shown that PCBs, by stimulating the cytochrome P-450 dependent mixed function oxidase system in the mammalian liver, can activate secondary carcinogens and mutagens or de-activate primary carcinogens and mutagens. Pretreatment with PCBs markedly increases liver injury by vinyl chloride, a known human liver carcinogen, but decreases injury by 1,1-dichloroethylene (274). PCBs promote the metabolism of dimethylnitrosamine to an active mutagen (281, 282), but de-activate a primary carcinogen (MNNG). PCBs are exceptionally efficient stimulators of the mixed function oxidase system and are now used routinely to activate suspect carcinogens in bacterial mutagenesis bioassays (276, 280).

PCBs are known to stimulate the mixed function oxidase system at extremely low doses (219) and their action in doing so is potentiated by other environmental pollutants such as BHC and DDT (246).

In addition to their direct carcinogenic effects and co-carcinogenic effects, the immunosuppressive effects of PCBs are also expected to be important in potentiating the action of other carcinogens.

III.15. Mutagenic and Teratogenic Effects

Green et al. (285) found no evidence of cytogenetic effects in bone marrow or spermatogonial cells of rats after treatment with single or multiple doses of Aroclors 1242 and 1254. There was a significant decline in the rate of cell division in rats treated with multiple doses (150-500 mg/kg/day for 4-5 days). Calandra (68) reported that a dominant lethal mutagenic study with Aroclors 1242, 1254, and 1260 in mice had given negative results, but details were not presented.

Villeneuve et al. (218) reported that single oral doses of 12.5, 25 and 50 mg/kg Aroclor 1254 administered to pregnant rabbits were fetotoxic, but did not induce teratogenic effects. Linder et al. (179) reported that Aroclors 1254 and 1260 had minor fetotoxic effects when administered at 100 mg/kg/day to female rats on days 7-15 of pregnancy; Aroclor 1254 also caused substantial mortality in pups prior to weaning. The pups were described as "grossly normal" but apparently were not autopsied.

Teratogenic effects and mutagenic effects of Aroclor mixtures have been reported in birds (141, 154, 155, 156, 157, 158, 159, 161). By analogy with PCDDs, PCDFs might be expected to be mutagenic and/or teratogenic. Hence it is regrettable that the studies in mammals have been reported incompletely. Except for that of Green et al. (285), none of the studies listed above can be regarded as satisfactorily negative.

III. 16 Effects in Humans

III. 16.1 Chloracne

Chloracne is an occupational disease associated with occupational exposure to PCBs, poly-chlorinated naphthalenes (PCNs), chlorinated phenols, 2,4,5-T, PCDFs, and perhaps polychlorinated diphenyl ethers. The etiology of the disease has been reviewed by Schulz (286), Crow (287), and Kimbrough (5).

Exposed workers develop small dermal cysts, pustules, and comedos. These are most commonly found on the face and ears, although other areas are commonly affected, including the abdomen, back, thighs, forearms, buttocks, and scrotum. This widespread distribution has been ascribed to direct contact of the affected parts with contaminated clothing. Melanosis and secondary inflammation may occur also. Systemic effects have also been stated to occur in several cases, perhaps due to inhalation of toxic vapors. These systemic effects include nausea, lassitude, anorexia, digestive disturbance, edema of the face and hands, abdominal pain, vomiting, burning and soreness of the eyes, impotence and hematuria (2,5). Deaths due to liver damage have also been reported in association with exposure to PCNs (2,5). Hepatic porphyria has been associated with exposure to 2,4,5-T (5), but Crow has suggested that these cases might in fact be attributable to exposure to chlorinated benzenes used in the same plant (287).

The onset of chloracne following initial exposure is usually quite slow. After occupational exposure to PCBs and PCNs, the latent period is typically about 7 months. The course of the disease is protracted. Chloracne usually persists for several months following removal from the source and may last up to four years (2).

The extent to which PCBs, and PCDFs, and other chemicals are responsible for chloracne is still not fully clear. PCDFs are said to be particularly toxic and acneigenic and to have been abandoned by industry for that reason (287). The symptoms arising from exposure to the other chemicals named above are said to be generally similar, except for the liver effects associated with PCNs. In the case of 2,4,5-trichlorophenol, Kimmig and Schulz, using an animal model, traced the acneigenicity primarily to contaminants, which they identified as PCDDs, especially 2,3,7,8-TCDD (288). Chloracne has been induced in human volunteers by skin application of TCDD and commercial PCNs (5, 286). However, Vos and Beems (204) reported that commercial PCNs are contaminated with PCDFs, as a result of the contamination of commercial naphthalene with dibenzofuran. As pointed out in Section II.5.5 above, commercial PCBs also are now known to contain trace quantities of PCDFs. Hence the relative roles of PCBs, PCNs, and PCDFs in causing chloracne are confused.

According to Crow, chloracne has been associated primarily with mixtures containing penta- and hexachloronaphthalene, and tetra, penta- and hexa-chlorobiphenyl. This suggests a structure-toxicity relationship parallel to that of the CDDs (148). Chloracne is now reported much less frequently than prior to 1944, and only one recent outbreak associated with manufacture of PCBs has been reported (5, 289). It is not clear whether the decline in cases associated with PCBs reflects improvements in industrial hygiene, reduction in PCDF contamination, or other factors. Epidemiological studies have recently been started (290).

No long-term follow-up studies of occupationally exposed workers have yet been reported, so there is no information on the carcinogenicity of PCBs.

III.16.2 Yusho

"Yusho" is a disease reported in Japan late in 1968 and traced to consumption of rice-oil contaminated with PCBs from a leaking heat-exchanger on 5-6 February 1968. Extensive reports on Yusho have been published by Japanese investigators and several summaries in English are available (35, 291-293). At least 1,291 persons were affected by the disease and descriptive epidemiology has been presented for 325 cases (291).

The contaminated rice-oil is reported to have contained about 1,000 ppm of PCBs (Kanechlor KC-400) and is now known to have contained also about 5 ppm of a mixture of CDFs (34, 35, ; Tables II.5.3, III.16.1). Thus the Yusho episode involved proportionately much greater exposure to CDFs than is normal for occupational exposure to PCBs or likely for environmental exposure to PCBs. This provides some interesting information about the effects of PCDFs on humans, but limits the value of the Yusho episode as a predictor of the effects of environmental exposure to PCBs.

A rough dose-response relationship was constructed relating severity of symptoms to estimated consumption of the oil (Table III.16.1). Severe cases were induced by consumption of less than 700 mg of PCBs (and less than 3.6 mg of PCDFs). According to Kuratsune (291) the smallest dose causing overt symptoms was about 500 mg of PCBs ingested over approximately 50 days by an individual weighing about 50 kg (i.e. about 200 µg/kg/day PCBs).

However, these figures are probably too high by a factor of 2-3, since they were based on an estimate of 2,000-3,000 ppm for the concentration of PCNs in the Yusho oil, whereas the most recent measurements place the average concentration at only 900-1,000 ppm (Table III.16.2).

Table III.16.1 (from ref. 291)

Relation between the amount of the K rice oil used by patients and their clinical severity.

Amount of oil	Non-affected		Light cases		Severe cases		Total	
	No.	%	No.	%	No.	%	No.	%
Less than 720 ml	10	(12.0)	39	(49.0)	31	(39.0)	80	(100.0)
720–1,440 ml	0	(0.0)	14	(31.0)	31	(69.0)	45	(100.0)
More than 1,440 ml	0	(0.0)	3	(14.0)	18	(86.0)	21	(100.0)

Table 3. PCDF's and PCB's in Kanechlors and Yusho oil.

		PCDF's (ppm)		PCB's (ppm)	
SAMPLE		PEAK HEIGHT METHOD	PERCHLORINATION METHOD	PEAK HEIGHT METHOD	PERCHLORINATION METHOD
KANECHLOR	300	1	1.5	-	-
	400	18	16.6	-	-
	500	4	2.5	-	-
	600	5	2.7	-	-
YUSHO OIL	A	5	4.4	830	870
	B	4	5.1	900	920
	C	5	5.2	1030	980

Table III.16.2 (from ref. 35)

Hence the minimal dose for overt symptoms would have been about 70-100 ug/kg/day of PCBs, together with about 0.4 µg/kg/day of PCDFs. The most highly exposed individuals would probably have ingested more than 3 µg/kg/day of PCDFs (Table III.16.1), a dose that is comparable with the lethal dose of 2,3,7,8-TCDF in guinea pigs (71). However, it is not clear that any individuals actually died as a direct result of Yusho (291).*

As in the case of chloracne, the onset of symptoms was delayed: reported cases peaked 3-7 months after the contaminated oil was shipped (291, Table III.16.3). Table III.16.4 summarizes clinical findings on 180 patients studied in detail. The general clinical findings were similar to those in industrial chloracne, but the Yusho patients had several additional symptoms. Outstanding among these were hypersecretion of the Meibomian gland, and hyperpigmentation of the skin; the latter was associated with increased melanization. Porphyrins were not measured in Yusho victims, but other signs of porphyria such as photosensitivity were not reported. Liver damage apparently was not marked, but there was elevation of serum alkaline phosphatase in severe cases, and increase in smooth endoplasmic reticulum in biopsied material (292). Other characteristic findings in Yusho were elevated blood-serum triglycerides, increased excretion of 17-ketosteroids in the urine, respiratory distress with secondary infections (292), and peripheral neuropathy (Table III.16.4). Unfortunately parameters of the hepatic mixed function oxidase system do not appear to have been measured.

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* One 25-year-old male admitted to hospital in June 1969 (one year after contracting Yusho) with severe abdominal pain and suspected perforated duodenal ulcer died from heart failure 3 weeks after operation (401).

Two Yusho victims who died in 1968-69 were autopsied. In addition to pathological skin changes, both had cystic dilatation, hyperplasia, and hyperkeratosis of the excretory duct of the esophageal gland. The 25-year-old male mentioned in the footnote to the previous page also had marked fibrinous pericarditis and multiple myocardial fibrosis with myofibrillar degeneration (401).

A number of live-born and at least 2 still-born infants were afflicted with Yusho (34, 291, 295). Their symptoms included hyperpigmentation of the skin, nails and gingivae, eye discharge, abnormally large fontanelles, swollen faces, and exophthalmos (259). The abnormal

Table III.16.3 (from ref. 291)

Distribution of patients with yusho diagnosed by January 20, 1969, by sex and month when symptoms appeared.

Month	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
Before 1968	2	1.3	2	1.2	4	1.2
1968						
January	0	0.0	0	0.0	0	0.0
February	4	2.5	6	3.6	10	3.1
March	8	5.1	12	7.2	20	6.2
April	15	9.5	10	6.0	25	7.7
May	18	11.4	15	9.0	33	10.2
June	23	14.6	33	19.8	56	17.2
July	27	17.1	32	19.2	59	18.1
August	34	21.5	30	18.0	64	19.7
September	17	10.8	12	7.2	29	8.9
October	8	5.1	14	8.4	22	6.8
November	1	0.6	1	0.6	2	0.6
December	1	0.6	0	0.0	1	0.3
Total	158	100.0	167	100.0	325	100.0

Table III.16.4 (from ref. 291)

. Percent distribution of symptoms of Yusho reported by 189 patients examined before October 31, 1968.

Symptoms	Males (N = 89)	Females (N = 100)
Dark brown pigmentation of nails	83.1	75.0
Distinctive hair follicles	64.0	56.0
Increased sweating at palms	50.6	55.0
Acnelike skin eruptions	87.6	82.0
Red plaques on limbs	20.2	16.0
Itching	42.7	52.0
Pigmentation of skin	75.3	72.0
Swelling of limbs	20.2	41.0
Stiffened soles in feet and palms of hands	24.7	29.0
Pigmented mucous membrane	56.2	47.0
Increased eye discharge	88.8	83.0
Hyperemia of conjunctiva	70.8	71.0
Transient visual disturbance	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Feeling of weakness	58.4	52.0
Numbness in limbs	32.6	39.0
Fever	16.9	19.0
Hearing difficulties	18.0	19.0
Spasm of limbs	7.9	8.0
Headache	30.3	39.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0

pigmentation gradually faded but growth was normal, in contrast to that of children poisoned post-natally, whose growth was retarded.

One infant born before its mother was poisoned apparently contracted Yusho via breast-milk (296).

Recovery from Yusho has been very slow. Table III 16.5 shows that there was little significant improvement during the first two years. By 1974, the dermal and mucosal symptoms that had been most marked at the beginning of the episode had gradually improved, while symptoms such as general fatigue, poor appetite, inconstant abdominal pain, heavy headedness and headache, feeling of numbness and pain at the limbs, cough and expectoration of sputum, have become more prominent year by year (297: Table III.16.6). These are considered to reflect some internal disturbances (35).

Serum triglyceride levels have remained high in males, but have started to decline in females (Table III.16.7). Poisoned women continued to give birth to affected children at least until 1972 (35). However, liver function tests have not yet given evidence of serious liver lesions (35).

A preliminary tabulation of deaths among Yusho patients (Table III. 16.8) suggests the possibility of an excess of cancer, especially in the stomach and liver, but there are too few cases as yet to draw conclusions.

The scanty data available suggest that tissue levels of PCBs in Yusho victims declined rapidly in the first year after their poisoning and are now only a few times higher than the average in the Japanese population (Table III.16.9). However, dermatological symptoms and other signs, including serum triglyceride levels, are correlated with concentrations of PCBs in the blood (Table III.16.10).

Table III.16.5 (from ref. 291)

Prognosis of patients with Yusho.*

Grade of clinical severity**	Clinical conditions			
	Improved	Stational	Worsened	Total
0	16 (66.7)	6 (25.0)	2 (8.3)	24 (100.0)
1	30 (51.7)	16 (27.6)	12 (20.7)	58 (100.0)
2	23 (52.3)	16 (36.4)	5 (11.4)	44 (100.0)
3	12 (52.2)	10 (43.5)	1 (4.3)	23 (100.0)
4	0 (0)	10 (100.0)	0 (0)	10 (100.0)
Total	81 (50.9)	58 (36.5)	20 (12.6)	159 (100.0)

* Calculated from the figures reported by Toshitani and Kitamura.

** 0: Physical complaints without skin-lesions.
1: Pigmentation of the skin and the mucous membrane.
2: Comedo formation.
3: Acneform eruptions.
4: Extensive distribution of acneform eruptions.

Table III.16.6 (from ref. 35)

Frequency of subjective symptoms complained by patients with Yusho from 1973 to 1974

Symptoms	Proportion[a] %
Fatigue	51.4
Headache	41.7
Phymata in articular region	8.3
Fever	2.8
Cough and sputum	56.9
Digestive disorder	40.3
Numbness of extremities	33.3
Menstrual disturbance	26.9 (7/26)

[a] Calculated by Kuratsune from original figures published by Koda and Masuda (ref. 4).

. Results of followup st[illegible]y on serum triglyceride levels in patients with Yusho[A].

Patients			Triglyceride (mg/100ml) Mean ± S.D.					
Sex	Age	No.	1969	1970	1971	1972	1973	1974
Male	11 - 73	14	159 ± 57	166 ± 55	169 ± 60	174 ± 69	164 ± 68	160 ± 118
Female	7 - 59	26	155 ± 75	161 ± 70	155 ± 80	153 ± 63	129 ± 50[B]	111 ± 56[B]

A : Cited from a report by Okumura et al. (ref.19).

B : Significantly lower than in 1969, 1970, 1971, and 1972 ($p < 0.05$).

Table III.16.7 (from ref. 35)

Table III.16.8 (from ref. 35)

Deaths seen among patients with Yusho[A].

CAUSE OF DEATH	NUMBER
MALIGNANT NEOPLASMS	9
STOMACH CANCER	2
STOMACH CANCER + LIVER CANCER	1
LIVER CANCER + LIVER CIRRHOSIS	1[B]
LUNG CANCER	1
LUNG TUMOR	1
BREAST CANCER	1
MALIGNANT LYMPHOMA	2
CEREBROVASCULAR LESION	3
AMYLOIDOSIS	1[B]
OSTEODYSTROPHIA FIBROSA	1[B]
MYOCARDIAL DEGENERATION + PERICARDITIS	1[B]
STATUS THYMICOLYMPHATICUS	1[B]
LIVER CIRRHOSIS	1
SUICIDE	1
SENILITY	1
TRAFFIC ACCIDENTS	3
TOTAL	22

A : CITED FROM A REPORT BY URABE 1974 (REF.2).
B : AUTOPSIED CASES.

Table III.16.9 (from ref. 35)

PCB's concentration in tissues of patients with Yusho and other diseases.

CASE	TIME OF DEATH, OPERATION	PCBS (PPM) SKIN WHOLE BASIS	SKIN FAT BASIS	ADIPOSE TISSUE WHOLE BASIS	ADIPOSE TISSUE FAT BASIS	LIVER WHOLE BASIS	LIVER FAT BASIS	REFERENCE
CASE 1 HIGH SCHOOL BOY	NOV. 1968			76 (FACE) 13 (ABDOMEN)				10
CASE 2,3 ADULT MALE, FEMALE	NOV. 1968			32,46 (CHEESE-LIKE SUBSTANCE FROM ACNEFORM ERUPTIONS)				
CASE 4 BOY, 13 YRS.	JULY 1969			1.3 (MESENTERY)	3.7	0.14	9.5	11,12
CASE 5 MALE 25 YRS.	JULY 1969	1.2	8.7	2.8 (MESENTERY)	15.1	0.2	10.4	
CASE 6 MALE 73 YRS.	NOV. 1969	1.0	4.4	3.8 (MESENTERY)	8.4	0.07	3.1	
CASE 7 FEMALE 48 YRS.	DEC. 1970	0.6	0.8	0.7 (MESENTERY)	0.9	0.07	1.3	
CASE 8 MALE 46 YRS.	MAY 1972	1.8	3.2	4.3 (MESENTERY)	6.5	0.08	8.4	
CASE 9 FEMALE 33 YRS.	SEPT. 1972			1.9 (SUBCUTANEOUS)	2.9			
CASE 10 MALE 72 YRS.	APRIL 1975			0.19 (MESENTERY)	0.4	0.04	3.0	14
NATIONAL SURVEY MALES AND FEMALES 25-49 YRS.	1973	0.04-1.7 (N=54)		0.2-4 (N=47)	0.3-6.4 (N=48)	0.01-0.6 (N=51)	0.2-3.1 (N=30)	13

Table III.16.10 (from ref. 35)

Prevalence of dermatological and other signs among patients with Yusho from April 1973 to March 1974, in connection with concentration and gaschromatographic pattern of PCB's in blood.

SIGNS		PREVALENCE (%)^{C}			
		GROUP A (43 CASES)	GROUP B (26 CASES)	GROUP C (3 CASES)	TOTAL (72 CASES)
PIGMENTATION	SKIN	51.2^{B}	0^{B}	0	30.6
	PALPEBRA	72.1^{B}	19.2^{B}	0	50.0
	GINGIVA	95.3^{B}	57.7^{B}	66.6	80.6
	NAIL	74.4^{B}	34.6^{B}	0	56.9
ACNEFORM ERUPTION		34.9^{B}	0^{B}	0	20.8
COMEDO		34.9	23.1	0	29.2
INFECTION OF SKIN		32.6	11.5	0	23.6
DEFORMATION OF NAIL		65.1^{A}	38.5^{A}	0	52.8
ALOPECIA		0	3.8	0	1.4
DISORDER IN TEETH		18.6	7.7	0	13.9
HYPERSECRETION OF MEIBOMIAN GLAND		93.0	80.8	100.0	88.9
PCB's IN BLOOD	CONC. (PPB) AV.±S.D.	7.2±4.9	4.3±3.1	1.7±0.2	5.9±4.5
	PATTERN	A^{D}	B^{D}	C^{D}	

A : SIGNIFICANT ($p < 0.05$) DIFFERENCE.

B : SIGNIFICANT ($p < 0.01$) DIFFERENCE.

C : CALCULATED BY KURATSUNE FROM FIGURES PUBLISHED BY KODA AND MASUDA (ref. 4).

D : "A" MEANS THE CHARACTERISTIC GASCHROMATOGRAPHIC PATTERN OF PCB's REMAINING IN THE BODY OF MOST PATIENTS WITH YUSHO. "B" MEANS GASCHROMATOGRAPHIC PATTERNS SOMEWHAT SIMILAR TO "A". "C" MEANS PATTERNS INDISTINGUISHABLE FROM THOSE OF NORMAL PERSONS.

An important new finding is that PCDFs have been retained in the tissues of Yusho victims, expecially in the liver where their ratio to PCBs is as high as 1:4 (Table III.16.11), versus 1:200 in the Yusho oil and 1:60,000 in the original Kanechlor mixture (Table III.16.2). The CDFs found in the tissues consist mainly of one penta- and one hexa-CDF: most of the isomers in the Yusho oil have not been retained (Figure III.16.1). The gas-chromatographic pattern of PCBs in Yusho victims also differs from that in ordinary persons (Figure III.16.2). Thus there appears to have been selective metabolism and/or excretion of certain CBs and CDFs in Yusho victims. The possibility that CDFs have been formed in the liver by metabolism should not be overlooked.

From the data available it is not possible to determine to what extent the persistence of symptoms in Yusho is due to tissue injury, persistence of PCBs in the body, and/or persistence of PCDFs in the body. The parallels between Yusho and chloracne are very close, and it is not clear to what extent the differences (such as the greater frequency of hyperpigmentation and eye discharge) are attributable to the high concentration of PCDFs in the Yusho incident.

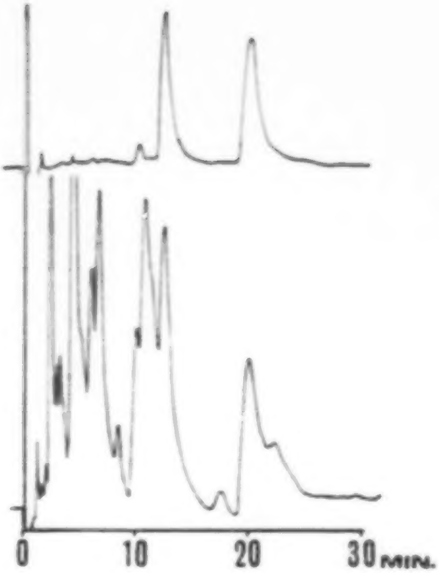
III.16.3 Animal models for Yusho and chloracne

Yusho and chloracne are closely related to two diseases in domestic animals. "Chick edema disease" has been discussed in Section III.6.2: the incident of contamination which caused the Yusho episode in 1968 also caused an outbreak of chick edema disease (144). "X-disease" in cattle, characterized primarily by hyperkeratosis, but also by diarrhea, poor appetite and symptoms of the mucous membranes, has been associated with exposure to PCNs and PCBs (5, 298). In each case it is likely that PCDFs are responsible for some of the toxic effects (298, Section III.6.2).

Table [illegible]. PCB's and PCDF's in tissues of patients with Yusho and ordinary persons.

SUBJECTS	TISSUE	CASE NO.	TIME OF DEATH	PCB'S (PPM) BASIS		PCDF'S (PPM) BASIS		RATIO PCB'S/PCDF'S	
				WHOLE	FAT	WHOLE	FAT	WHOLE	FAT
YUSHO PATIENTS	ADIPOSE	1	1969	1.4	3.4	0.013	0.03	108	113
		2	1969	1.3	8.5	0.006	0.04	21[illegible]	213
		3	1972	1.2	2.1	0.007	0.01	171	210
		AV.		1.3	4.7	0.009	0.03	144	157
	LIVER	1	1969	0.05	4.7	0.025	2.3	2	2
		2	1969	0.06	5.6	0.010	1.1	6	5
		3	1972	0.03	3.5	0.003	0.3	10	12
		AV.		0.05	4.6	0.013	1.2	4	4
ORDINARY PERSONS	ADIPOSE	1	1975	1.0	1.4	ND	ND		
		2	1975	0.4	0.7	ND	ND		
	LIVER	1	1975	0.08	1.3	ND	ND		
		2	1975	0.02	1.0	ND	ND		

Table III.16.11 (from ref. 35)

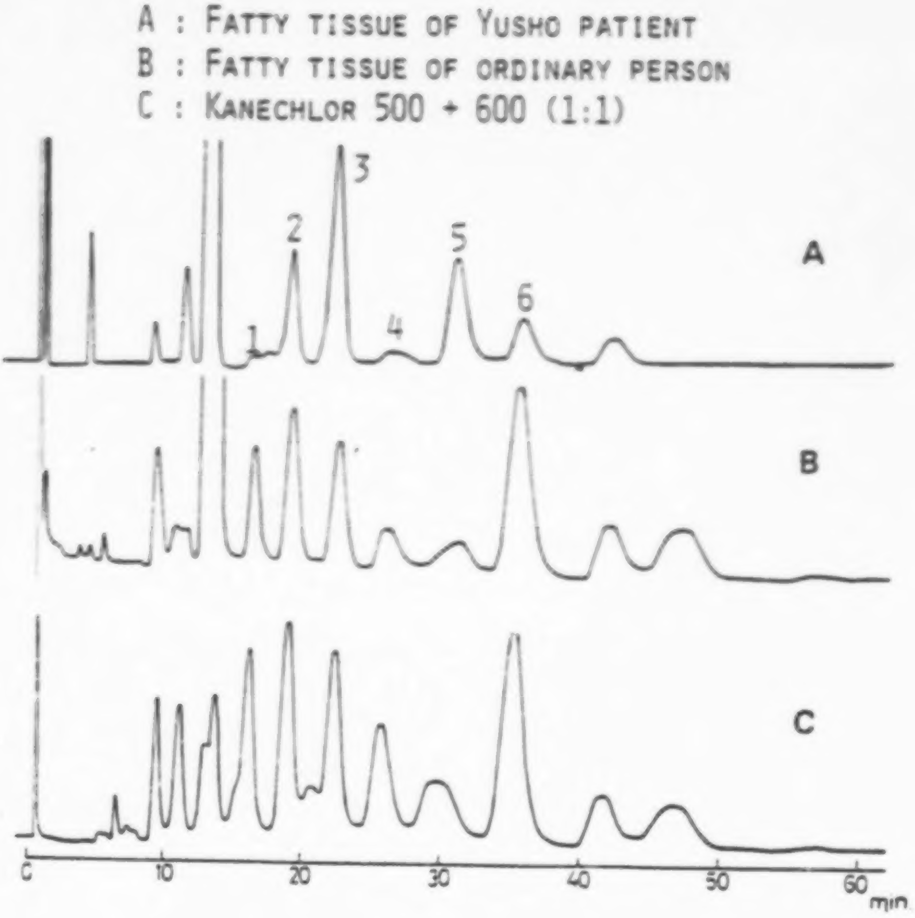


UPPER :
PCDF FRACTION FROM
LIVER OF YUSHO PATIENT.

LOWER :
PCDF FRACTION FROM
YUSHO OIL.

Gaschromatograms of PCDF fractions from liver of patient with Yusho and from "Yusho oil."

Figure III.16.1 (from ref. 35)



Gaschromatograms (ECD) of PCB's on SE-30.

Figure III.16.2 (from ref. 35)

Some traditional laboratory animals have proved to be poor models for Yusho and chloracne. Rodents in particular seem to be relatively resistant both to PCBs and to PCDFs (Sections III.6 - III.10 above) and some of the symptoms induced in rodents, such as hepatic porphyria, are not prominent in affected humans. However, hairless mice fed Yusho oil developed skin lesions, including hyperkeratosis of the hair follicle and sweat gland and cystic dilatation (298). These symptoms appeared more severe than those in mice exposed to Kanechlor KC-400 (210), suggesting a possible effect of PCDFs. However, mice were unaffected by doses of 2,3,7,8-TCDF as high as 6 mg/kg (71), 100 times higher than the cumulative doses of PCDFs ingested by Yusho victims. Rats also were affected by Yusho oil, showing hyperkeratosis of the epidermis and hair follicles, parakeratosis of epidermis, and hyperplasia and keratotic metaplasia of the ductal epithelium in the Meibomian glands of the eyelids (403). These symptoms parallel those of the Yusho victims, but have not been reported in other experiments with rats.

Rabbits have been used as a model for screening acneigenic agents (5, 129, 288). Rabbits are very sensitive to toxic-effects of PCDFs (129) and application to rabbit skin causes chloracne-like symptoms (288). Vos and Beems (204) found that the rabbit skin test was a sensitive bioassay for PCDF contamination in PCB mixtures, but as discussed above a later experiment suggested that individual PCB isomers also have acneigenic action.

Recent work with rhesus monkeys suggests that this species is the best model for chloracne and Yusho (69, 194-198). The symptoms found in rhesus monkeys and their offspring fed low doses of PCBs are quite similar to those found in humans. It is not yet clear whether the severe gastric ulceration found in monkeys (and to a lesser extent in other species, but not in rodents) has a parallel in humans, but the long persistence of digestive disturbances reported in victims of Yusho and chloracne suggests that it may do so. The occurrence of cystic dilatation and hyperplasia of the duct of the esophageal gland in two autopsied victims of Yusho (401) is a close but not exact parallel to the findings in monkeys.

III.16.4 The role of PCDFs in the toxicity of PCBs to humans

A key question in formulating criteria for human exposure to PCBs is the extent to which PCDFs or other contaminants may be responsible for the toxicity of commercial PCBs or environmental residues. Since many of the symptoms of chloracne and Yusho are known to be produced in humans (287) or animal models (205, 129, 299, 288) by PCDFs and PCDDs, it could be hypothesized that PCDFs are the primary or exclusive toxic agents. However, as pointed out in Section III.8 above, PCB mixtures with extremely low levels of PCDFs have caused chloracne in monkeys, and apparently pure chlorobiphenyl isomers have done so also (197). Hence it seems more probable that some chlorobiphenyls -- perhaps isomers with 3,4,3',4'-substitution or other structural similarity to 2,3,7,8-TCDF -- or their metabolites share the toxic properties of PCDFs. In any case, since PCDFs are present in commercial mixtures (Section II.5.5) and appear to be formed from them in the environment (Section II.8.3), in service (Section II.8.4) and by metabolism (Appendix E), it is difficult and probably academic to attempt to distinguish their effects completely in weighing the environmental hazards posed by PCBs.

IV. ENVIRONMENTAL FATE AND EFFECTS

IV.1. Persistence, Metabolism and Fate

IV.1.1. Persistence

PCBs have a long life in the environment (1-3). The more chlorinated compounds in particular are resistant to metabolism (Appendix E) and persist in soils (305), water (41, 42), sediments (50, 99, 105) and biological tissues (111, 130, 141, 155, 170, 208, 35) for weeks, months or even years. Indirect evidence for their persistence in the environment is provided by their wide distribution, even in remote parts of the earth where PCBs are unlikely to have been used (1-3, 56, 225, Appendix C).

Precise data on the life-time of PCBs after release into the environment are difficult to obtain, because natural systems are open: if a decline in concentrations is observed it is difficult to distinguish degradation from net export. Hom et al. (329) provided almost the only unequivocal data: they found PCBs in dated (varved) sediments in the Santa Barbara basin off southern California. PCBs were first detectable in sediment layers dated to the mid-1940's and their concentration in the overlying sediments increased steadily in parallel with the known increase in PCB usage. This shows that PCBs can last for at least 30 years in these circumstances (anaerobic sediments with no burrowing organisms). More recently Risebrough et al. (56) have identified PCBs in Antarctic snow at depths up to 6 metres below the surface.

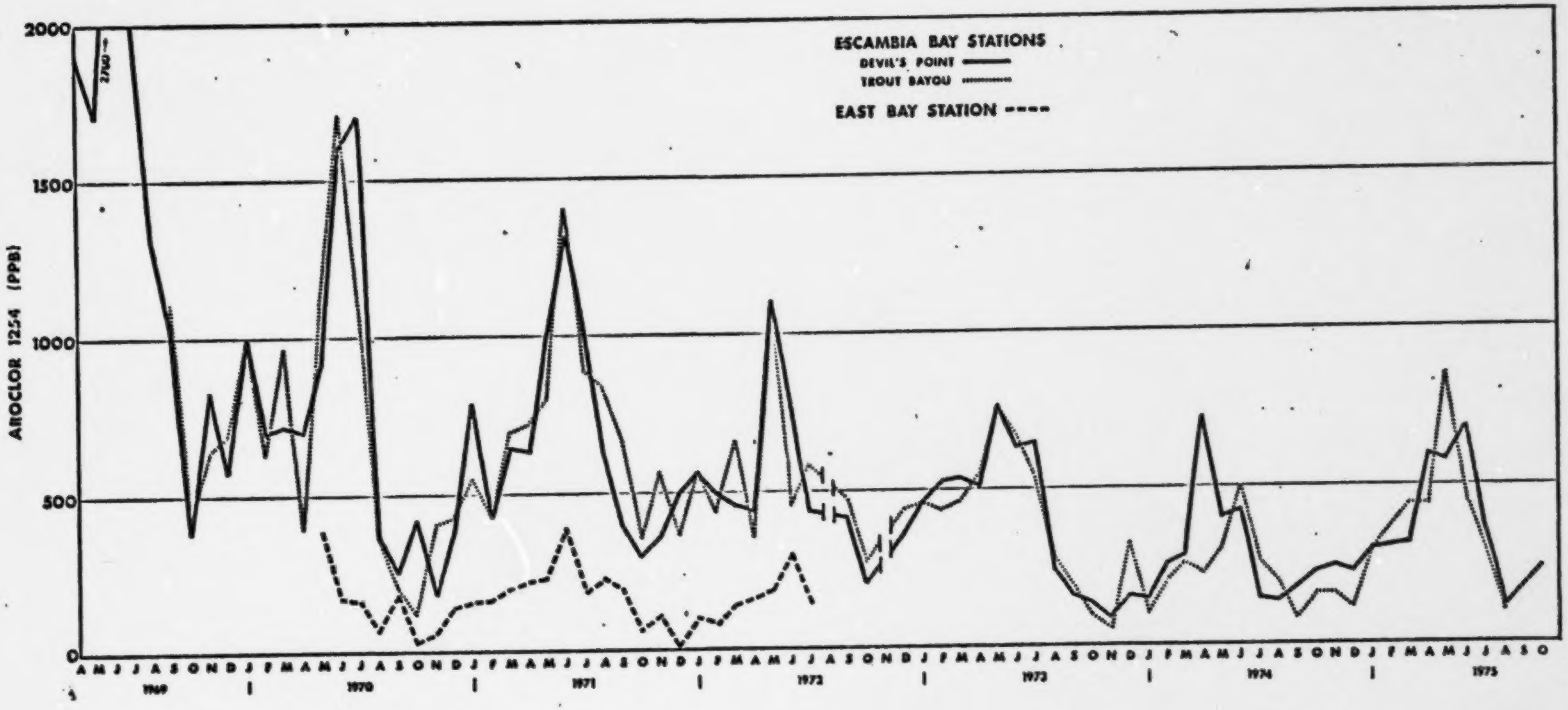
Figure IV.1.1 shows concentrations of PCBs measured in oysters in Escambia Bay, Florida, since 1969 (330). PCBs in Escambia Bay are believed to have originated largely from a single point source, identified and closed in 1969 (99, 330). The residues in oysters in the bay show seasonal fluctuations (related to spawning) but otherwise have declined only slowly over the years since 1969.

All these data indicate that the more chlorinated PCBs have a life-time of years, if not decades, in at least certain compartments of the environment (2, 331).

IV.1.2. Differential Persistence of Lower and Higher Chlorinated Biphenyls.

Nisbet and Sarofim (332, 2) pointed out an anomaly, *viz.*, that most PCBs found in environmental samples consisted of penta- and higher CBs, whereas a substantial fraction of the PCBs released into the environment in the past must have consisted of tetra- and lower CBs. They suggested that the discrepancy was probably due to differential degradation of the lower CBs, ruling out the main alternative explanation (differential mobility) primarily because samples of PCBs from remote areas also consisted primarily of higher PCBs. A critical set of observations was that of Veith and Lee (333, 334), who showed from samples of water and fish from the Milwaukee River that the proportion of lower CBs declined as the water and sediments moved downstream away from the major industrial sources. However, this decline could be explained either as differential volatilization or differential degradation (333).

RESIDUES OF AROCLOR 1254 IN OYSTERS



Attachment 1

Figure IV,1.1 (ref. 330)

The differential persistence and/or bioaccumulation of lower and higher CBs is important in establishing criteria, because it implies differential exposure of human and other target organisms to the various components of the various commercial mixtures. Accordingly the following sections of this document will pay particular attention to differences in environmental behavior between lower and higher CBs. The potential for human exposure to tetra- and lower CBs is of especial importance, because the main difference between Aroclors 1016 and 1242 is the lower proportion of the more persistent penta-CBs in the former (Table II.3.1). In 1972 it was believed that most human exposure was to penta- and higher CBs, but recently more evidence for human exposure to tetra-CBs has been presented (331, 332).

IV.1.3. Metabolism

Studies of the metabolic transformation and degradation of PCBs are summarized in Appendix E. Bacteria are able to metabolize biphenyl and mono- and di-CBs fairly rapidly, but tri- and tetra-CBs are degraded more slowly and penta-CBs hardly at all (Section E.1). Accordingly there is considerable differentiation of PCB mixtures during microbial degradation, as illustrated in Figures E.4 to E.7. Figure IV.1.2 (from ref. 13) shows that even 14 days' incubation with activated sludge had little effect on the higher components (mostly tetra-CBs) in Aroclor 1016.

Aquatic invertebrates and fish are able to metabolize tri-CBs to a substantial degree but have very little ability to metabolize tetra- or penta-CBs to non-polar products (Sections E.2 and E.3).

AROCLOR 1016 BIODEGRADATION

SEMI-CONTINUOUS ACTIVATED SLUDGE

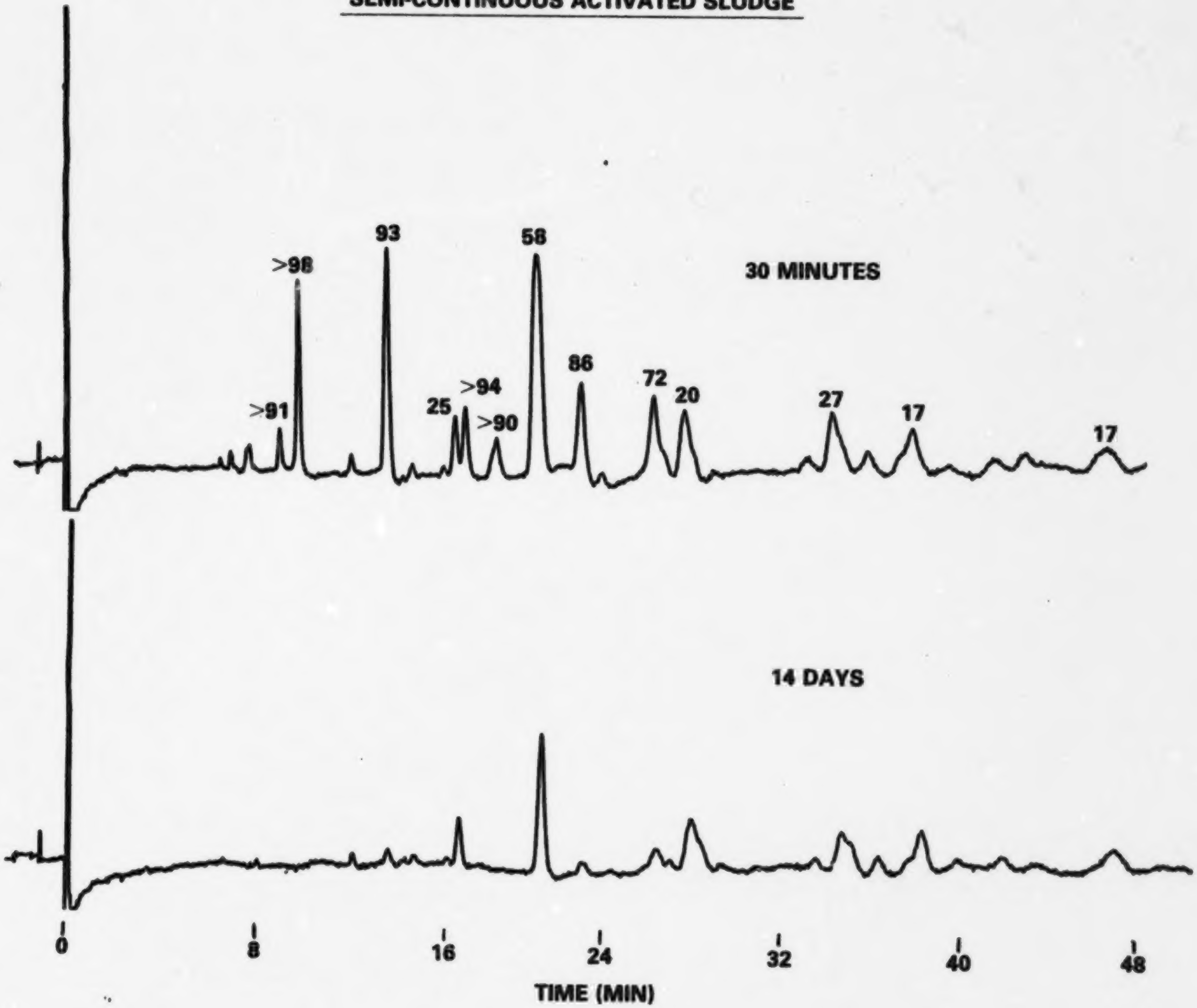


Figure IV.1.2

Birds and mammals can metabolize tetra- and penta-CBs to hydroxy derivatives at varying speeds, but they have very little ability to metabolize hexa- or higher CBs (Sections E.4 and E.5).

The principal mechanism of metabolism appears to be via oxidation to arene oxide (epoxide) intermediates, followed by rearrangement ('NIH shift') to form hydroxy-CBs. Hydroxylation at the 3- or 4-positions is favored (Section E.6). Less important mechanisms of metabolism are direct hydroxylation, dechlorination, and isomerization. There is evidence for metabolic formation of CDFs in chickens and rats (Section E.7). Unidentified non-polar metabolites are stored in invertebrates and fish (Section E.3).

Metabolism does not always represent detoxification, since CDFs are much more toxic and at least one hydroxy-CB is substantially more toxic than the parent compound. The formation of arene oxide intermediates is of much concern because this reactive class of compounds is implicated as causative agents in toxic, carcinogenic, and mutagenic effects (Section E.8).

IV.1.4. Photodegradation

The photochemical properties of PCBs were summarized in Section II.8. Photolysis of PCBs has been studied only under laboratory conditions and it is difficult to use the data to predict rates of degradation in the environment. In the experiment which most nearly simulated environmental conditions (61), there was little overall degradation after 3 weeks in sunlight and the principal net effect was a shift from higher to lower CBs in the mixture (Table II.8.3).

Photodegradation does not necessarily represent detoxification, since lower CBs are formed by dechlorination and small quantities of CDFs are sometimes formed (Sections II.8.2, II.8.3).

IV.1.5. Transport

PCBs are mobile in the environment and may be transported in solution, by motion of suspended sediments, as vapors, on airborne particulates, or in the tissues of mobile animals (2, 12, 332, 333, 47, 50, 52, 53, 54, 56, etc.). They are concentrated at the air/water interface (52, 55) and may be transported across it by several mechanisms, including volatilization, solution, dry fallout of particulates, precipitation in rain and snow, or ejection in spray (Section II.8). Critical measurements are scanty and conflicting (56) and it is still not possible to construct satisfactory models of transport to and from the aquatic environment (56, 331).

Within bodies of water, there is evidence that the transport of PCBs is controlled by the presence and transport of sediments (Section II.6.5). The presence of sediments or particulates inhibits volatilization of PCBs (41, 42) and is strongly associated with their transport (50, 56, 12). In natural waters with high sediment loads it may be reasonable to treat PCBs as passively transported on particulates, but it is not known whether this would be valid for the ocean or for clear lakes. However, it is questionable whether conditions in aquaria without sediments provide good models for natural environments.

IV.1.6. Fate

Theoretically, there are three ultimate fates for PCBs released into the environment: metabolic degradation, photolytic degradation, and deposition in sediments in lakes or the deep ocean (2). All three processes are known to occur, but their relative importance in the natural environment remains to be determined (2, 56, 331).

IV.2. Bio-accumulation and Bio-magnification

IV.2.1. Mechanisms of uptake and accumulation

One of the most important environmental properties of PCBs is their tendency to be "bio-accumulated" by aquatic organisms -- i.e., to be concentrated into their tissues to levels much higher than those in the ambient water (1-3). This property results from the high solubility of PCBs in lipids and their low solubility in water: Metcalf *et al*. (45) have shown that the degree of bio-accumulation of a chemical in aquatic animals is closely related to its partition coefficient between organic solvents and water (Figure II. 6.6). There is a further tendency for PCBs to be concentrated into the tissues of animals to levels higher than those in their food (1-3): this phenomenon is sometimes referred to as "bio-magnification". Within organisms, PCBs are further concentrated into certain organs, especially the fat (1-3).

The kinetics of organochlorine compounds in animals have recently been reviewed by Moriarty (335). It is necessary to consider bio-accumulation as a multi-stage process: the chemicals are taken into the organism via food, water, or air, circulated through the organism in the blood, transferred into and out of various organs, and finally metabolized and/or excreted. Each stage of transfer is a dynamic process whose rate depends on the concentrations of the chemicals in the various media and body organs. Under conditions of constant exposure, an organism may eventually reach a quasi-equilibrium, in which the concentrations of the chemical in the tissues are constant. It is then possible to define a storage factor (ppm in organism/ppm in ambient medium). In principle, storage factors should be defined separately for each organ in the body, but in practice it is common to define a single "bio-accumulation factor" (average ppm in whole body/ppm in ambient water). However, where the time required to reach equilibrium is long, as it often is for PCBs, the organism's physiological state may change and a true equilibrium may never be reached (335).

Aquatic plants take up PCBs directly from water, and their bio-accumulation factors reflect partitioning across the cell membranes. Animals may be exposed to PCBs in food, in air, or in water. Aquatic invertebrates and fish have to process so much water in order to breathe that they usually reach quasi-equilibrium with PCB concentrations in water fairly quickly; intake via food is usually only a minor route of intake (336). Accordingly the storage factor (ppm in whole body/ppm in ambient water) is the most useful measure of bio-accumulation. However, for air-breathing animals such as birds and mammals the food is usually the most important route of intake and the storage factor (ppm in whole body/ppm in food) is the most useful measure: this factor is sometimes referred to as the "bio-magnification factor" (336).

Laboratory experiments on bio-accumulation and bio-magnification are usually highly simplified, involving constant exposure via one route only. The situation in the real world is more complex, since animals are exposed simultaneously via several routes, levels of exposure fluctuate greatly in time and space, and the animals' physiological state changes in response to aging and periodic environmental stresses. Accordingly, while laboratory experiments provide useful information on mechanisms of uptake and excretion, and comparative data for the various components of PCBs, they do not necessarily provide quantitative measures of the degree of bio-accumulation and bio-magnification to be expected in the field. Measurements on wild animals in the field are needed to gauge the extent of bio-accumulation and bio-magnification, and to specify their variability in time and space.

IV.2.2. Bio-accumulation in Aquatic Invertebrates

In laboratory experiments, Sanders and Chandler (98) found that uptake and bio-accumulation of Aroclor 1254 by some aquatic invertebrates was very rapid. Bio-accumulation factors for various organisms ranged from 2,800 in stone-flies to at least 47,000 in *Daphnia magna* (Table IV.2.1). In scud (*Gammarus pseudolimnaeus*), the tri- and tetra-CBs in Aroclor 1254 were differentially accumulated by factors 2-8 times higher than the hexa- and hepta-CBs (Table IV.2.2). Nebeker and Puglisi (93) found bio-magnification factors of 16,000-36,000 for

Table IV.2.1
(from ref. 98)

Biological magnification of ^{36}Cl-labeled Aroclor® 1254 by aquatic invertebrates

Organism	Organisms per sample[1/]	Water concentration ppb $\bar{x}$ ± SE[2/]	Organism concentration (4 day exposure) ppm x ± SE	Magnification factor[3/] 1-day	4-day	7-day	14-day	21-day
Daphnid *Daphnia magna*	60	1.1 ± 0.2	52 ± 2.0	24,700	47,000	-	-	-
Phantom midge *Chaoborus punctipennis*	5	1.3 ± 0.1	30 ± 1.6	22,000	23,000	23,800	24,800	-
Scud *Cammarus pseudolimnaeus*	6	1.6 ± 0.1	39 ± 3.0	17,000	24,000	26,000	27,500	27,000
Mosquito larvae *Culex tarsalis*	10	1.5 ± 0.3	27 ± 2.0	12,600	18,000	20,000	-	-
Glass shrimp *Palaemonetes kadiakensis*	3	1.3 ± 0.1	16 ± 2.6	10,300	12,300	13,700	14,200	16,600
Stonefly *Pteronarcys dorsata*	3	2.8 ± 0.8	7.0 ± 0.30	2,100	2,500	2,800	2,900	2,800
Dobsonfly *Corydalus cornutus*	3	1.1 ± 0.1	5.1 ± 0.23	1,400	4,600	5,700	6,600	6,800
Crayfish *Orconectes nais*	2	1.2 ± 0.1	0.2 ± 0.20	570	1,700	3,400	4,500	5,100

[1/] Samples were taken in triplicate.

[2/] Samples were taken in triplicate and expressed as mean value ± standard error (P=.05).

[3/] Concentration in organism/concentration in water.

Table IV.2.2
(from ref. 98)

Changes in isomer ratios of Aroclor® 1254 residues in scud

Peak No.	Rel. Ret. Time[1]	No. Cl's[2]	Concentration Factor[3] Sample 1	2	Average	Aroclor® 1254 R.P.A.
1	0.27	3	2.00	2.00	2.00	0.03
2	0.37	4	1.69	1.72	1.71	0.29
3	0.45	4	1.81	1.81	1.81	0.11
4	0.50	4	1.80	2.00	1.90	0.05
5	0.63	5	1.31	1.31	1.31	0.51
6	0.77	5	1.00	1.00	1.00	1.00
7	0.98	5	1.13	1.16	1.15	0.38
8	1.07	5	1.07	1.16	1.12	0.57
9	1.19	6	0.33	0.66	0.49	0.03
10	1.31	6	0.84	0.85	0.85	0.65
11	1.59	6	0.55	0.55	0.55	0.33
12	1.67	6	0.80	0.80	0.80	0.30
13	1.79	7	0.43	0.57	0.50	0.07
14	2.04	6	0.56	0.60	0.58	0.45
15	2.61	6	0.50	0.62	0.56	0.08
16	3.22	7	0.25	0.50	0.37	0.04
17	3.68	7	0.25	0.25	0.25	0.04

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[1] Retention time relative to p,p' DDE on GLC column of 2 mm i.d. x 1.8 m 0.3% w/w OV-7 on 80-100 mesh Corning® 110 glass beads; 15 ml/min. flow of nitrogen carrier gas; column operated at 155° C.

[2] Number of chlorine atoms substituted on biphenyl ring determined by gas chromatography-mass spectrometry (9).

[3] Concentration factor = Relative Peak area of sample ÷ Relative Peak areas as Aroclor® 1254.

the same species of scud when exposed to Aroclor 1242, and of 28,000-108,000 when exposed to Aroclor 1248. In estuarine shrimps, bio-accumulation factors ranged up to 26,000 during chronic exposure to Aroclor 1254 (104: Table IV.2.3). In short-term tests, shrimp accumulated Aroclor 1016 to levels 2-4,000 times higher than those in the ambient water within 96 hours (100). Bio-accumulation factors reported in oysters ranged up to 101,000 (108) and to 165,000 (337).

In a comparative study in a model ecosystem, Metcalf *et al.* (45) found higher bio-accumulation factors for representative tetra- and penta-CBs than for a representative tri-CB (Tables E.4 and E.5). In snails the bio-accumulation factors (E.M. in Table E.5) recorded after 33 days were 5,800 for the tri-CB, 39,400 for the tetra-CB, and 59,600 for the penta-CB. In mosquito larvae the corresponding factors were 815, 10,600, and 17,300, but these represented only 6 days' exposure. It should be noted, however, that some of the unidentified non-polar metabolites were bio-accumulated even more than the parent CBs (Table E.4). "Unknown I" from the tri-CB, for example, was bio-accumulated 12,000 times in the snail and 8,000 times in the mosquito.

IV.2.3. Bio-accumulation in Fish

In laboratory experiments in flowing sea water, juvenile pinfish (*Lagodon rhomboides*) and spot (*Leiostomus xantherus*), both accumulated increasing amounts of Aroclor 1254, up to quasi-equilibrium levels, as duration of exposure increased (111). Spot exposed to 1 ppb Aroclor 1254 for 56 days reached maximum residue levels of

Table IV.2.3

ACCUMULATION OF AROCLOR 1254 IN Palaemonetes pugio WITH TIME

AFTER EXPOSURES TO THE CHEMICAL IN WATER AT THREE CONCENTRATIONS (μg/1)
(Each value represents a composite sample of 10 animals)

Length of Exposure (hr/ days)	Control		0.04		0.09		0.62	
	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor
0	0.1	*	0.1	*	0.1	*	0.1	*
1	---	*	0.1	*	0.1	*	0.1	*
2	---	*	0.1	*	0.1	*	0.1	*
3	---	*	0.1	*	0.1	*	0.1	*
4	---	*	0.1	*	0.1	*	0.1	*
8	---	*	0.1	*	0.1	*	0.12	190
12	---	*	0.1	*	0.1	*	0.14	230
16	---	*	0.1	*	0.1	*	0.26	420
24 / 1	---	*	0.1	*	0.1	*	0.20	320
36 / 1.5	---	*	0.1	*	0.1	*	0.20	470
48 / 2	---	*	0.1	*	0.10	1100	0.37	600
72 / 3	---	*	0.1	*	0.14	1560	0.58	930
96 / 4	---	*	0.1	*	0.15	1670	0.40	650
154 / 6.5	0.1	*	0.1	1590	0.33	3670	1.28	2060
336 / 14	---	*	0.13	3250	0.43	4780	7.40	11930
504 / 21	0.1	*	0.15	3750	0.45	5000	6.67	10900
672 / 28	0.14	*	0.17	4250	1.57	17400	10.82	17450
840 /35	0.10	*	0.21	5250	0.75	8330	16.48	26580
			PCB -	STOPPED				
1176 / 49	0.1	*	0.1	*	0.12	*	3.24	*
1512 / 63	0.15	*	0.1	*	0.13	*	1.64	*

* Magnification factor not calculated.

37,000 times the water concentration in 14-28 days (Figure IV.2.1), whereas pinfish exposed to Aroclor 1254 accumulated it to 22,000 times the water concentration (111). Thereafter, the concentration of PCB (ppm in tissues) remained roughly constant while the total amount continued to increase as the fish grew. After placing the fish in clean water the PCBs were gradually eliminated, falling by half in about 4 weeks (Figure IV.2.1). In a parallel experiment with pinfish exposed to Aroclor 1016, a bio-accumulation factor of about 20,000 was measured after 42 days (Table IV.2.4, Figure IV.2.1, ref. 100). Thus the bio-accumulation of Aroclors 1016 and 1254 was similar in this fish. However, there was some differential uptake of the components of Aroclor 1016, since the peaks 1-3 in the chromatogram (mono- and di-CBs) were proportionately reduced in the PCBs stored in the fish tissue, whereas the tri- and tetra-CBs were proportionately increased in the fish tissues (Figure IV.2.2, and Table IV.2.5; cf. Figure II.3.3).

DeFoe _et_ _al_. (113) found that the bio-accumulation factor for adult fathead minnows at 25°C is approximately 120,000 for Aroclor 1248 and 270,000 for Aroclor 1260. Female fathead minnows accumulated about twice as much PCBs as the males but the difference is largely due to the greater amount of lipids in the females. The residues of PCBs in the fish tissues were directly proportional to the water concentration (Figure IV.2.3). The storage factor for fish lipids (ppm in lipids/ppm in water) was between 1 and 2 million (_ibid_.). Nebeker _et_ _al_. (112) found that the bio-accumulation factor

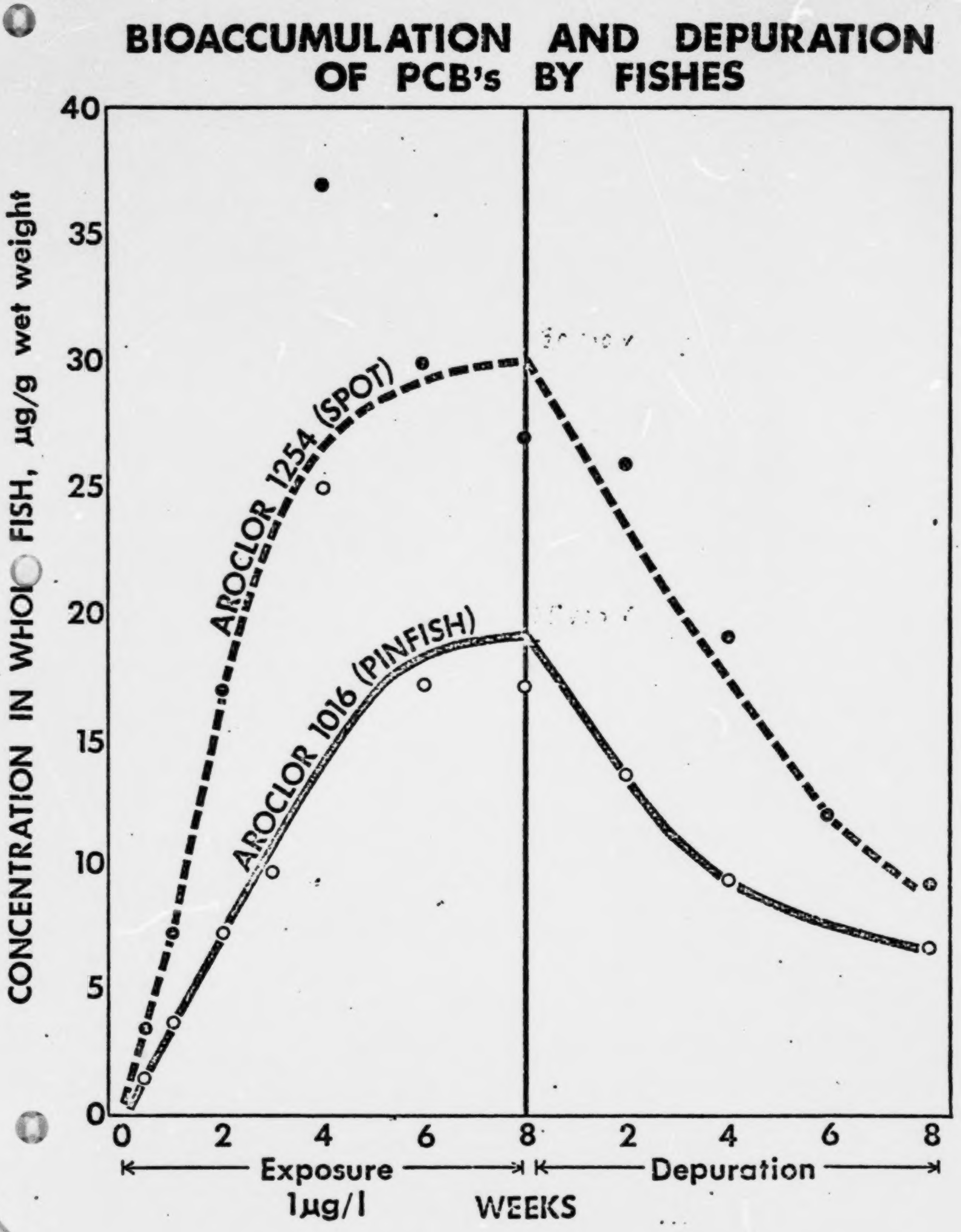


Figure IV.2.1
(from ref. 330)

Table IV.2.4
(from ref. 100)

Toxicity and Uptake of Aroclor 1016 by Pinfish (*Lagodon rhomboides*) Exposed for 42 Days in Three Separate Experiments

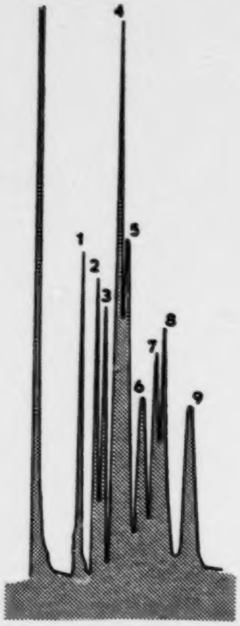
Test concentration (μg/liter)		Mortality (%)	Concentration in fish (μg/g, wet weight)		
Nominal	Measured		Flesh	Flesh and skin	Whole fish
Control	ND[a]	36	ND	ND	ND
0.1	ND	16	0.7	0.8	2.4
1.0	0.8	48	5.1	6.3	11
10.0	3.0	38	60	90	166
Control	ND	12	0.5	0.6	0.5
1.0	0.9	16	4.0	6.0	17
3.2	2.5	16	34	39	65
10.0	7.0	28	63	76	170
32.0	13	44[b]	140	180	620
Control	ND	6	ND	ND	ND
10.0	6.8	6	23	49	111
32.0[c]	21	50[b]	30	48	106
100.0[c]	59	50[b]	38	72	205

[a] ND, not detectable: <0.2 μg/liter in water: <0.2 μg/g in tissue.

[b] Mortality significantly greater than in control fish, $\alpha = 0.01$.

[c] Exposure terminated and tissues analyzed when 50% of the fish died: 33 days at 32 μg/liter and 18 days at 100 μg/liter.

Figure IV.2.2 (from ref. 100)



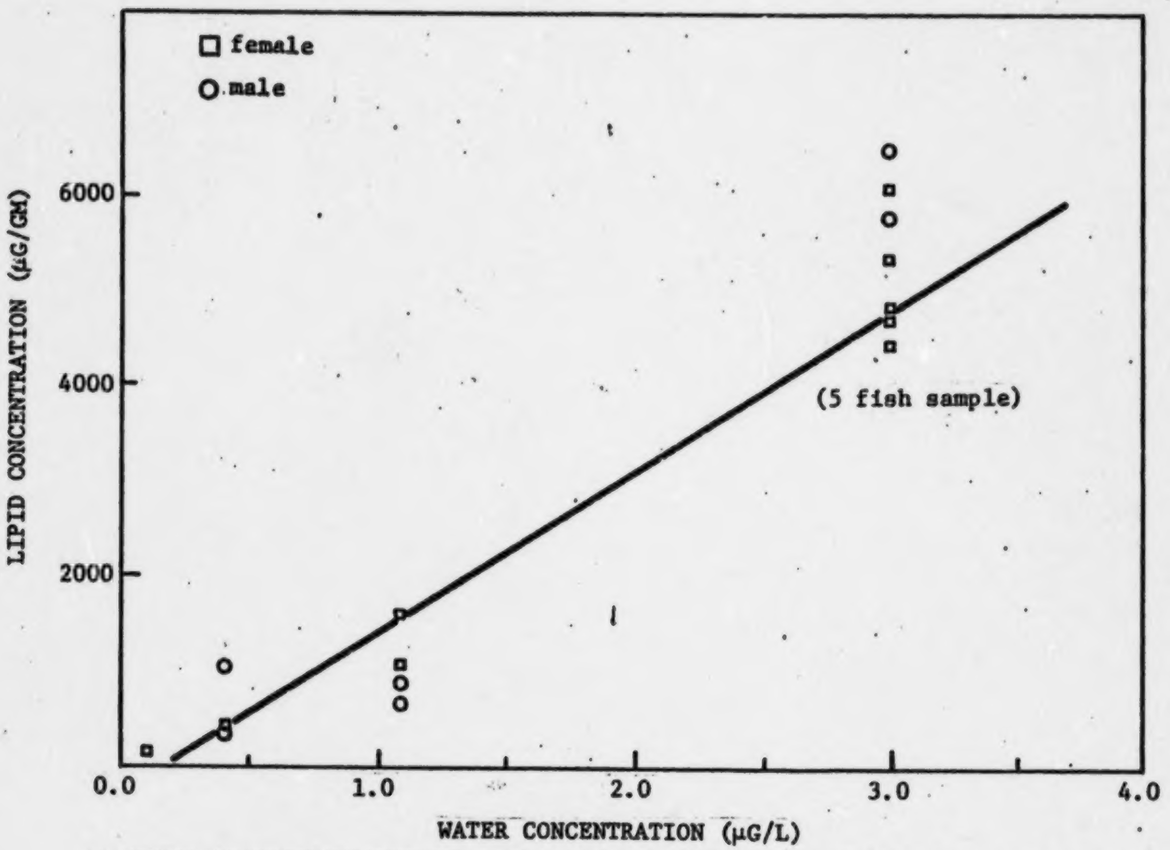
Chromatogram of 9 peaks of Aroclor 1016 reference standard. Operating conditions: gas flow nitrogen 25 ml/min; injection and detector temperature 210°C; oven temperature 190°C; ^{3}H electron capture detector; 152.4 × 0.32 cm glass column packed with 2% OV-101 on 100–120 Gas Chrom Q.

Table IV.2.5 (from ref. 100)

Percentages of the 9 Measured Peaks from Chromatograms of Aroclor 1016 Reference Standards and from Tissues of Pinfish Exposed to 1 μg/liter of Aroclor 1016 for 56 Days and then Held in PCB-free Water for 56 Days

	Percentage of peak[a]									Chromatograms
	1	2	3	4	5	6	7	8	9	
Reference standard	12.4	11.3	10.0	23.5	13.3	6.4	8.1	8.8	6.2	8
All tissues through 14 days of exposure	2.4	5.3	1.8	39.6	7.7	10.3	12.1	9.4	11.4	7
All tissues, days 21–56 of exposure	2.5	5.0	2.1	33.2	7.8	11.9	11.3	10.3	15.9	12
All tissues during 56 day depuration	1.0	1.7	0.3	38.2	2.8	15.0	12.4	11.9	16.7	7

[a] Determined as $\frac{\text{peak height}}{\text{sum of nine peaks}} \times 100$.



Source: DeFoe, D.L., G.D. Veith, and R.W. Carlson, 1975. Effects of Aroclor 1248 and 1260 on the fathead minnow (Pimephales promelas), U.S. Environmental Protection Agency, National Water Quality Laboratory, Duluth, Minnesota.

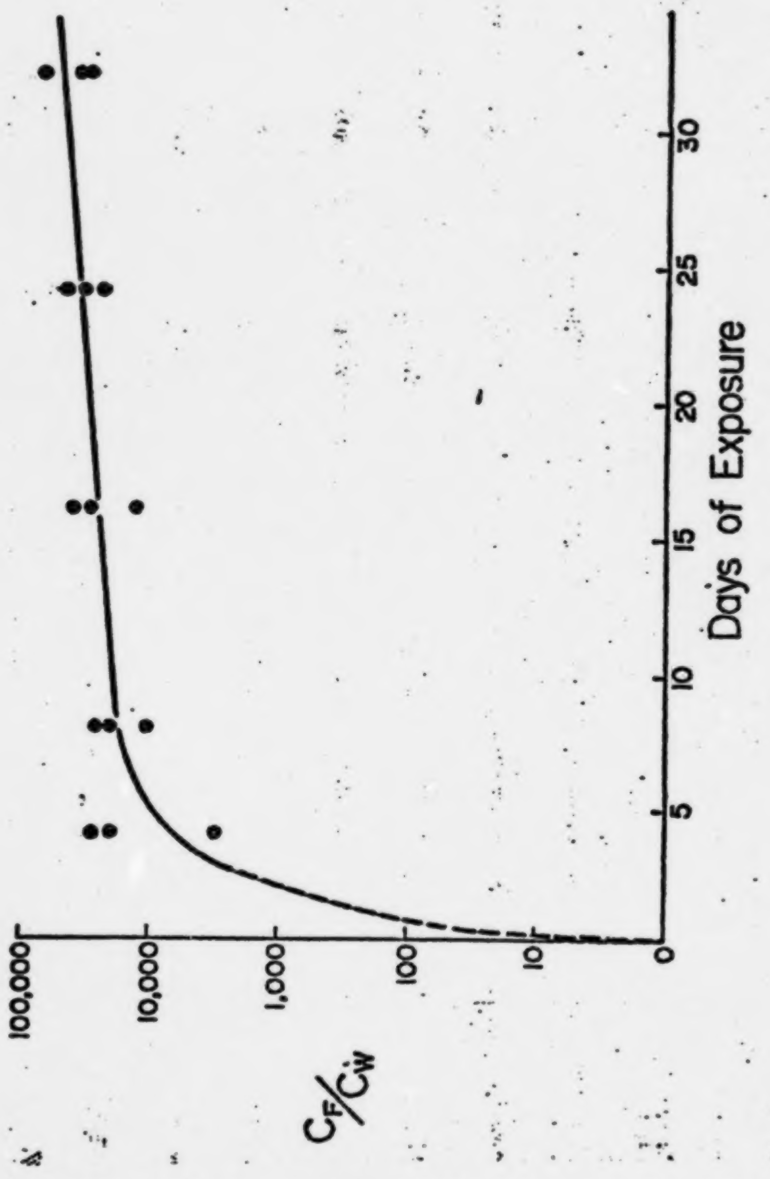
Figure IV.2.3 (ref. 113)
Aroclor 1248 residue in fathead minnows, normalized to lipid concentration in fish.

for Aroclor 1254 in fathead minnows was between 110,000 and 240,000, and that for Aroclor 1242 was between 32,000 and 274,000. Although the results varied somewhat between replications of these experiments there is no evidence that the bio-accumulation factors in this fish increase significantly with the degree of chlorination of the mixture. Veith and Kiwus (338) conducted an experiment with fathead minnows exposed to Aroclor 1016 and found a bio-accumulation factor of about 50,000, but the concentrations in the fish were still rising after 32 days (Figure IV.2.4). This result for Aroclor 1016 falls well within the range observed for the same species for Aroclor 1242, and within a factor of 3 of that observed for Aroclors 1254 and 1248 (112, 113).

Bio-accumulation factors for bluegills exposed to Aroclors 1248 and 1254 were in the range 26,300 to 71,400. No major modification of the PCB isomer ratios were observed in the tissue residues, and no new compounds were identified (95).

Several studies have shown that fish also store PCBs when fed contaminated food while kept in clean water. In these cases the biomagnification factors were quite modest, because the fish were able to exchange PCBs across their gills with the ambient water; in natural environments both water and food would contain PCBs and fish would take in PCBs via both routes.

Coho salmon (Oncorhynchus kisutch) fed Aroclor 1254 for 240 days at dietary concentrations of 0.4 to 580 ppm (14.5 to 14,500 µg/kg body weight per day) accumulated whole body residues which



Variation of the Aroclor 1016 bioconcentration factor (C_F/C_W) with time of exposure. The C_F is derived from composites of five fish.

Figure IV.2.4 (ref.338)

were 0.9 to 0.5 times the dietary levels (95). The highest residue value was 300 ppm.

Accumulation and retention of Aroclor 1254 by rainbow trout (Salmo gairdnerii) from the diet followed the same pattern as that for coho salmon (339). The relative concentration of PCB in the lipid fraction of trout increased rapidly during 8 weeks' exposure to 15 ppm Aroclor 1254 in the food, then tended to equilibrate at about 95 ppm (Figure IV.2.5). While the relative amount in the fish reached quasi-equilibrium, the absolute quantities continued to increase as the trout grew (Figure IV.2.6). The distribution of PCB among the tissues was dependent on the lipid content of the various tissues (Table IV.2.6).

The uptake of pure PCB isomers by fish has been studied in three experiments. Gruger et al. (121) fed 3,4,3',4' tetra-CB and two hexa-CBs to juvenile coho salmon at a dietary concentration of 3.3 ppm each (total 10 ppm) for 165 days. The material fed during the first 24 days was retained well in the tissues, but tissue concentrations tended to level off and after 28 days the concentration of the tetra-CB was only about half that of either of the two hexa-CBs (Tables IV.2.7, IV.2.8). The experiment is complicated by the fact that the treated fish lost weight and depleted their lipid reserves, and that equilibrium concentrations in the tissues had not been reached (Table IV.2.7). As in the case of the trout, most of the PCBs were concentrated in the lipid-rich tissues, and the average concentration of PCBs in lipids was similar (about 70 ppm) in all

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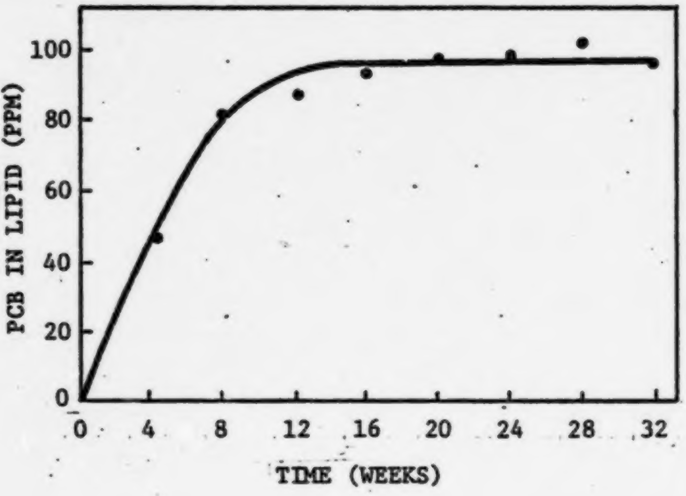

Source: Lieb, A.J., D.D. Bills and R.O. Sinnhuber, 1974. Accumulation of dietary polychlorinated biphenyls (Aroclor 1254) by rainbow trout (Salmo gairdneri), Journal of Agricultural and Food Chemistry, 22(4): 638-642.

Figure IV.2.5 (ref. 339)
PCB in lipid fraction of rainbow trout after exposure to 15 ppm Aroclor 1254.

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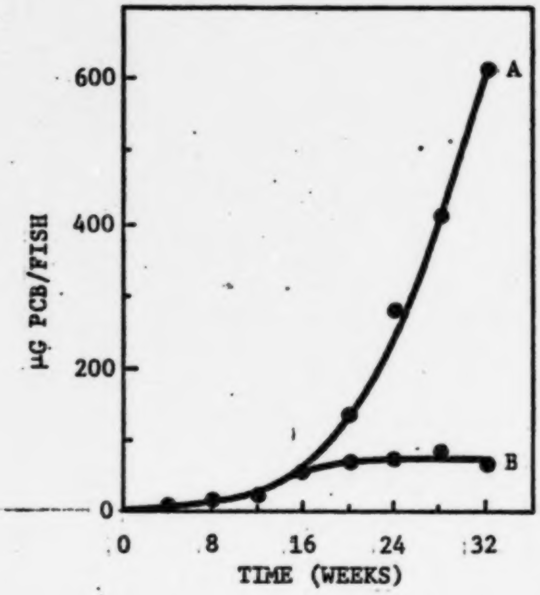

Source: Lieb, A.J., D.D. Bills, and R.O. Sinnhuber, 1974. Accumulation of dietary polychlorinated biphenyls (Aroclor 1254) by rainbow trout (Salmo gairdneri), Journal of Agricultural and Food Chemistry, 22(4): 638-642.

Figure IV.2.6 (ref. 339)
Total amount of PCB per fish: (A) fish on diet containing 15 ppm of PCB; (B) fish removed from diet containing 15 ppm of PCB at end of 16 weeks.

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Table IV.2.6 DISTRIBUTION OF AROCLOR 1254 IN TISSUE OF RAINBOW TROUT

Tissue	% lipid in tissue	Concn of PCB in lipid, ppm	Concn of PCB in tissue ppm
Visceral adipose	92.8	111	103
Gill	9.7	113	11.3
Muscle	2.7	104	2.8
Stomach	6.5	104	6.8
Liver	3.5	57	2.3
Whole fish	8.5	96	8.2

Source: Lieb, A.J., D.D. Bills, and R.D. Sinnhuber, 1974. Accumulation of dietary polychlorinated biphenyls (Aroclor 1254) by rainbow trout (*Salmo gairdneri*) *Journal of Agricultural and Food Chemistry* 22(4): 638-642.

Table IV.2.7 (ref. 121)

ANALYSES OF WHOLE JUVENILE COHO SALMON WHEN FED FOR 24, 53, AND 108 DAYS[1]

Group	Days	Body wt, g	Lipid content, wt%	3,4,3',4', chlorobiphenyl	2,4,5,2',4',5' chlorobiphenyl	2,4,6,2',4',6' chlorobiphenyl	Total
				Wet tissue;[2] µg/g			
Test	24	4.24	7.60	0.49	0.73	0.66	1.88
				(47%)	(70%)	(63%)	(60%)
	53	5.26	6.48	0.59	0.93	0.90	2.42
				(30%)	(47%)	(45%)	(41%)
	108	3.06	3.8	0.65	1.41	1.42	3.48
				(16%)	(35%)	(35%)	(29%)
Control	24	3.79	5.83	0.059	0.090	0.050	0.199
	53	5.42	7.38	0.044	0.047	0.053	0.144
	108	7.87	6.8	0.107	0.023	0.030	0.160

1. Three fish per group. 2. Percent of chlorobiphenyl amount found in fish relative to amount fed is given in parentheses.

Source: Gruger, E.H., Jr., N.L. Karrick, A.I. Davidson and T. Hruby. 1975. Accumulation of 3,4,3',4'-tetrochlorobiphenyl and 2,4,5,2',4',5'-and 2,4,6,2',4',6'-hexachlorobiphenyl in juvenile coho salmon. Environmental Science and Technology 9(2): 121-127.

Table IV.2.8 (ref. 121)

CONCENTRATIONS OF CHLOROBIPHENYLS AND LIPID CONTENT IN TISSUES OF JUVENILE COHO SALMON FROM TEST AND CONTROL GROUPS

Tissue specimens at 117 days	Lipid content, wt%	3,4,3',4' chlorobiphenyl	2,4,5,2',4',5' chlorobiphenyl	2,4,6,2',4',6' chlorobiphenyl	Total
		Wet tissue, μg/g			
Test group					
Brain	7.1	0.15	0.31	0.38	0.84
Liver	2.9	0.25	0.35	0.50	1.1
White muscle	2.6	0.29	0.63	0.64	1.6
Intestines	4.3	0.30	0.79	0.82	1.9
Stomach and pyloric caneca	3.3	0.43	0.76	0.95	2.1
Spinal column	6.6	0.92	1.4	1.4	3.7
Heart	-	0.98	1.2	1.5	3.7
Lateral line muscle	6.1	0.77	1.8	1.9	4.5
Spleen	-	0.85	2.0	2.1	4.9
Adipose	73.0	10.6	19.3	18.8	48.7
Control group					
Brain	6.6	0.020	0.009	0.017	0.046
Liver	3.9	0.034	0.014	0.037	0.085
White muscle	5.5	0.028	0.015	0.025	0.068
Intestines	6.6	01098	0.032	0.069	0.20
Stomach and pyloric caneca	4.2	0.032	0.014	0.022	0.069
Spinal column	23.7	0.096	0.026	0.032	0.15
Heart	-	0.85	0.068	0.18	1.1
Lateral line muscle	11.4	0.10	0.058	0.077	0.24
Spleen	-	(2.0)	0.13	0.12	(2.3)
Adipose	73.5	0.30	0.19	0.36	0.85
at 165 days					
Test Group					
White muscle		0.086	0.20	0.22	0.51
Brain		0.26	0.37	0.56	1.2

the tissues tested after 117 days (Table IV.2.8). After 48 days' starvation the concentrations of PCBs in the tissues were depleted somewhat (to a total of 33 ppm in adipose), but those in the brain were increased slightly (Table IV.2.8). The tetra-CB was excreted only slightly faster than the hexa-CBs (121).

Sanborn et al. (307) exposed green sunfish to three representative chlorobiphenyls in two pulsed exposures (24 hours' exposure to 1 or 2 ppb 9 days apart). Although the tri-CB was rapidly excreted and was almost eliminated after 15 days, the penta-CB was well retained and there was no evidence of elimination in the 15-day period of study (Figure IV.2.7). The tetra-CB was taken up and retained slightly less well than the penta-CB, but the concentrations in the fish at the end of the 15-day experiment were only 2-3 times smaller than those of the penta-CB, despite the limited exposure time (Figure IV.2.7). Thus the major difference in uptake and retention appeared to be between the tri- and tetra-CBs.

Metcalf et al. (45) exposed mosquito fish to the same three chlorobiphenyls under model ecosystem conditions (exposure via both food and water). Although the exposure to the fish was for only 3 days, they nevertheless accumulated the tri-CB to 6,400 times the water concentration, and the tetra- and penta-CBs to about 12,000 times the water concentration (Table E.5). Thus under these circumstances there was no difference in bio-accumulation between the tetra- and penta-CBs.

To summarise, under laboratory conditions fish bio-accumulate PCBs to concentrations 30,000 to 300,000 times higher than those in

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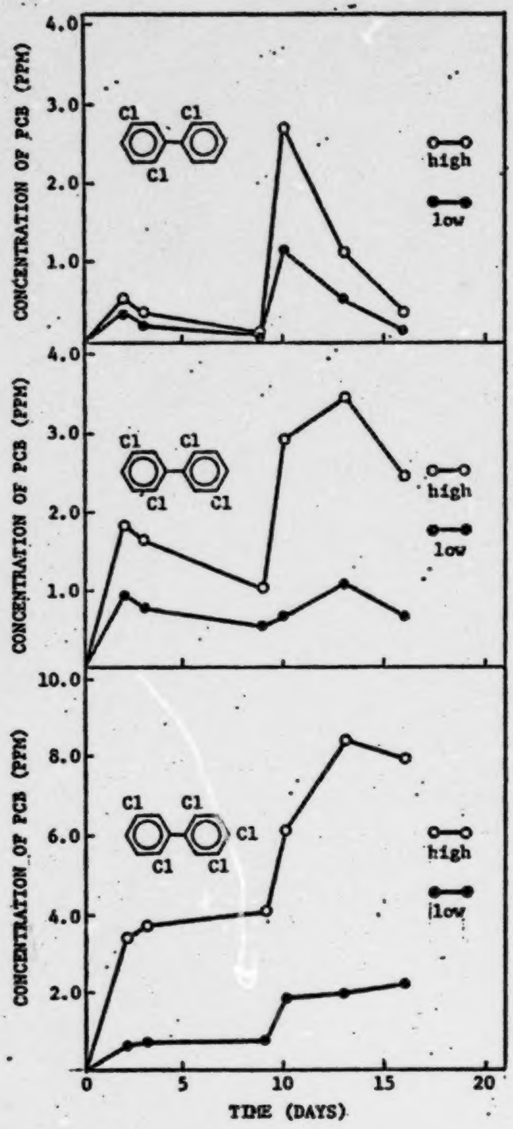


Source: Sanborn, J.R., W.F. Childers, and R.L. Metcalf, 1975. Uptake of three polychlorinated biphenyls, DDT, and DDE by the green sunfish, Lepomis cyanellus, RAF., Bulletin of Environmental Contamination and Toxicology, 13(2): 209-217.

Uptake of tri-, tetra-, and pentachlorobiphenyl by the green sunfish.

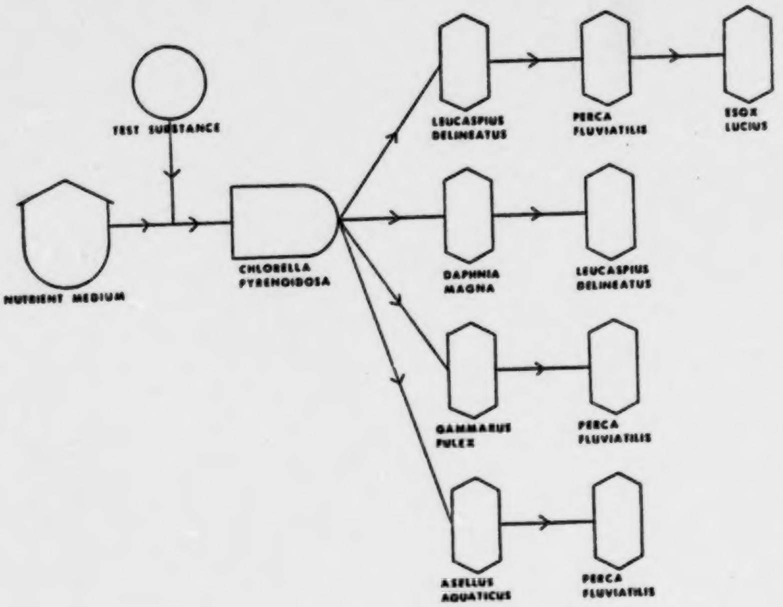
Figure IV.2.7

the ambient water (up to 2 million times higher in lipids). There is evidence that mono-, di-, and tri-CBs are poorly retained in fish tissues, but the differences in retention between tetra- and higher CBs are small, no more than a factor of 2-3 in the circumstances of these experiments.

IV.2.4. Bio-accumulation in Model Aquatic Ecosystems

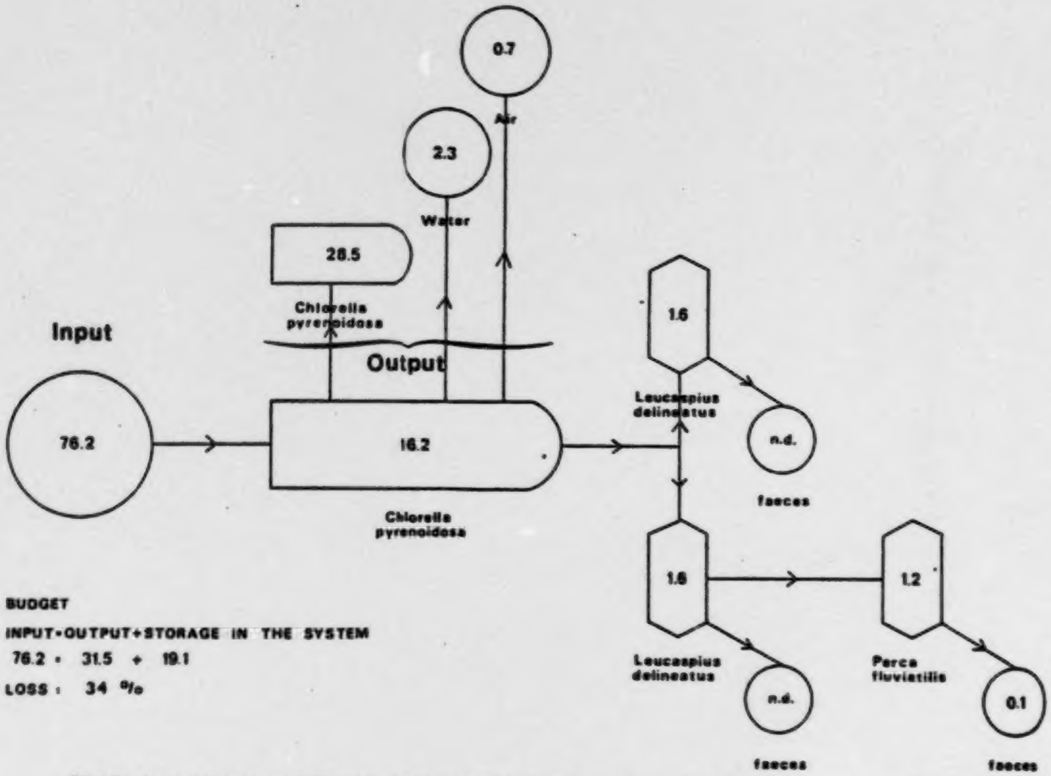
Two studies have been published of the behavior of PCBs in model aquatic ecosystems, designed to simulate the transport of PCBs from water through simple food chains. In one study by Metcalf *et al*. (45), radiolabelled chlorobiphenyls were applied to a mixed terrestrial-aquatic system and subsequently traced in water and various aquatic organisms. The results have already been summarized in the previous sections and in Appendix E (Tables E.3-E.5). The study showed how PCBs and their metabolites move from the terrestrial to the aquatic environment and are taken up there by aquatic organisms, including plants, insects, fish, and snails. Because the fish were only introduced into the system for the last few days of the experiment, the results do not indicate the full potential for bio-accumulation in food-webs: the figures of 6,000-60,000 for bio-accumulation factors in snails (Table E.5) give the best measure of this potential.

While Metcalf's system is primarily designed to investigate storage and biodegradability (45), a system designed by Södergren was designed to trace the movement of PCBs through a simple food-web (340). Figure IV.2.8 shows the basic design of the system and Figure IV.2.9 shows the results of a test with Clophen A50. Most of



Principle design of the model aquatic ecosystem. The organisms not mentioned in the text constitute alternative food-chains viable in the system, but not included in this study.

Figure IV.2.8
(from ref. 340)



Distribution and budget of Clophen A 50 within the model aquatic ecosystem (µg). n.d. = no substance detected.

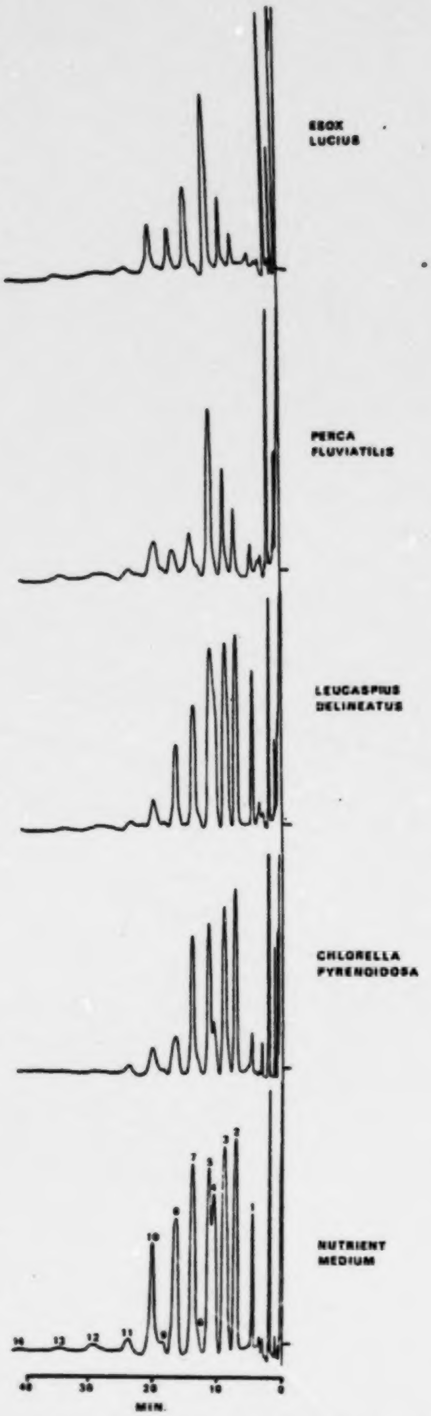
Figure IV.2.9
(from ref. 340)

the transport of the material to the first consumer organism (the cyprinid fish Leucaspius delineatus) took place via the alga Chlorella pyrenoidosa. There were some changes in the relative proportions of the components of the Clophen mixture as they passed through the system (Figure IV.2.10). However, these changes were very minor except in the two predatory fish (the pike Esox lucius and the perch Perca fluviatilis). Even the first component in the Clophen mixture (2,5,2',5'-tetraCB) was passed essentially unchanged through the alga to the first consumer (Figure IV.2.10).

IV.2.5. Storage and Bio-magnification in Birds

Storage and bio-magnification in birds are of interest for two reasons: (a) to indicate the range of residues likely to be ingested by humans eating wild or domestic birds; (b) to indicate the degree to which passage of residues up the food chain can lead to toxic effects in the birds themselves or in their predators.

In a study with chickens (149, 150), laying females fed Aroclor 1248 at 0.5 ppm accumulated 3.1 ppm in their adipose tissues and 0.22 ppm in their eggs after 8 weeks; correspondingly higher levels in tissues and eggs were found at higher feeding levels (Table IV.2.9). Similar results were obtained with Aroclor 1242: hens fed at 5 ppm laid eggs containing 1.7 ppm after 6 weeks (152). However, laying hens may be a poor model for other birds because their continuous egg-laying provides them with an important route of excretion (341) not available to wild birds which lay few eggs. Pheasants given 12.5 mg Aroclor 1254 weekly (equivalent to about 50 ppm in the



Gas chromatograms obtained from various components in a model aquatic ecosystem when testing Clophen A 50.

Figure IV.2.10
(from ref. 340)

Table IV.2.9
(from ref. 150)

—Transfer of PCBs[1] from the laying diet to the adipose tissues and eggs of hens

Diet no.	PCBs in diet	PCBs in adipose tissues			PCBs in eggs				
		1 wk.	4 wks.	8 wks.	1 wk.	4 wks.	6 wks.	7 wks.	8 wks.
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1 (Diet A)	0	0	0	0	0	0	0	0	0
2	0.5	1.54	2.16	3.10	0.10	0.16	0.21	0.20	0.22
3	1.0	2.21	4.41	6.62	0.19	0.33	0.42	0.45	0.41
4	10.0	10.4	22.7	37.1	1.05	2.21	2.83	3.11	3.06
5	20.0	16.3	54.7	82.7	2.19	4.51	5.37	5.72	7.04

[1] Aroclor 1248, Monsanto. Inc., St. Louis, Mo.

diet) for only 16 weeks accumulated an average of 24 ppm in their whole bodies; this did not decline significantly even after 6 months on a clean diet (141). Mallards fed 25 ppm Aroclor 1254 laid eggs containing 33-56 ppm PCBs (wet weight) (131). Ring doves fed 10 ppm Aroclor 1254 in the diet accumulated 8 ppm in the muscle, 5.5 ppm in the brain, 15.3 ppm in the liver, 736 ppm in the fat, and 4.8 ppm in the eggs (342, 161-163); after starvation the PCBs were mobilized from the fat and the birds died with 293 ppm in the brain (Table IV.2.10). American Kestrels fed 10 ppm Aroclor 1254 in the diet laid eggs with average residues of 225 ppm (dry weight, corresponding to about 30 ppm, wet weight) (164).

A number of workers studying the storage of PCBs in birds have reported that the lower chlorobiphenyls in Aroclor 1254 (tetra-CBs) are retained less well than the higher components (141, 131, 342, etc.). Call et al. (343) have provided quantitative data showing that tetra- and penta-CBs are stored at relatively lower levels and hexa- and hepta-CBs at relatively higher levels in Japanese quail than in the Aroclor 1260 with which they were fed.

However, the differential storage of PCBs in birds is not related simply to the degree of chlorination. DeFreitas and Norstrom (27), studying the elimination of components of Aroclor 1254 from pigeons, found that the components which were excreted and/or metabolized most rapidly were those with 2,3,- 3,4-, or 2,3,6- substitutions in at least one ring. Components with 2,4,5-, 2,3,4-, and 2,3,4,5- substitution patterns were not eliminated. Components with 2,5-

Table IV.2.10
(from ref. 161)

Organ levels, ppm, wet weight basis.

	Muscle	Brain	Liver	Fat
Pre-stress (n=4)	8.1±2.3 (4.1–9.9)	5.5±1.6 (4.8–8.0)	15.3±12.6 (3.6–27.5)	736.1±211.6 (481.2–1069.4)
Post-stress (n=5)	172.9±38.3 (120.0–227.0)	293.0±27.6 (254.0–340.2)	1118±267 (937.3–1688.0)	***

Figures are means, standard deviations, and range.
*** No fat present.

Table IV.2.11
(from ref. 344)

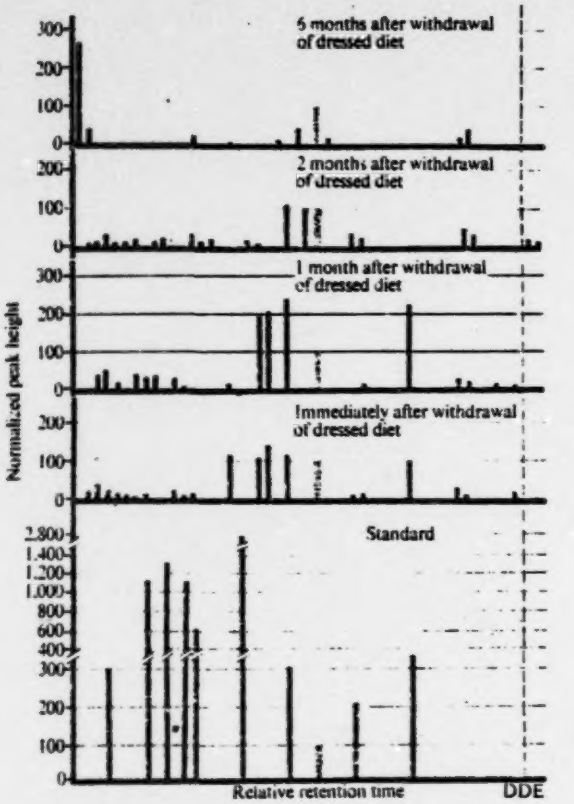
[redacted]

Mean Total PCB Residues (p.p.m.), found in Quail Tissue after Feeding 'Aroclor 1242' and 'Aroclor 1254' at 250 p.p.m. for 20 Days

Tissue	'Aroclor 1242'	'Aroclor 1254'
Liver	17.0 ± 2.6*	28.1 ± 7.9
Heart	3.2 ± 0.7	7.0 ± 1.1
Brain	7.8 ± 1.7	7.7 ± 2.3
Omental fat	123 ± 17	304 ± 31

* All figures are mean of six birds ± s.e.

35



Gas-liquid chromatographic "spectra", normalized to a common peak (stippled column) of 'Aroclor 1242' standard and mean pigeon liver residues following feeding at 500 p.p.m. in diet for 28 days.

Figure IV.2.11
(from ref. 344)

substitutions were eliminated, but much more slowly than those with 2,3-, or 3,4- substitutions. Bush et al. (155) obtained similar results for differential elimination of components of Aroclor 1254 from laying hens, and suggested that 4,4' substitution is more important than number of chlorine atoms in determining the degree of persistence of chlorobiphenyls in hens. This agrees with the findings of Matthews and Anderson (328) in rats.

Finally, Bailey and Bunyan (344) found that although Aroclor 1242 was not accumulated so strongly from the diet by Japanese quail as Aroclor 1254, the difference was only by a factor of 2-2.5 (Table IV.2.11). Although the lower components in Aroclor 1242 (di- and some tri-CBs) were not well retained in pigeon tissues, some tri- and most tetra-CBs were well retained and were only slowly excreted over a 2-6 month period (Figure IV.2.11). Of the components well retained in the pigeon tissues, only one (probably 2,5,3',4'-tetra-CB) is not present in Aroclor 1016 (Figure II.3.3). Thus, although there is substantial differentiation of the components of PCB mixtures as they are taken up, stored, and eliminated by birds, some tetra- and even tri-CBs are reasonably well retained in tissues and secreted into eggs.

Very little information is available on bio-magnification of PCBs by any fish-eating birds, which are of interest as being the species at greatest risk in the environment. However, cormorants (Phalacrocorax carbo) dosed with an average of about 300 ppm Clophen A60 (about 25 mg/kg/day) accumulated 850-2,750 ppm PCB residues in their whole bodies in 55-125 days, including residues of 10-20,000 ppm in their fat (142). This indicates a somewhat higher degree of bio-magnification than for any of the other birds listed above.

IV.2.6. Storage and Bio-magnification in Mammals

The storage and kinetics of PCBs in mammals are of interest (a) to indicate the range of residues likely to be ingested by humans eating domestic mammals; (b) as models for the storage and kinetics of PCBs in humans themselves. There is an extensive literature on the uptake, kinetics, and elimination of PCBs in mammals, but for this document primary interest is attached to the results of long-term feeding at low levels.

Absorption of PCBs from the gastrointestinal tract appears to be very efficient. Albro and Fishbein (345) fed nine individual chlorobiphenyl isomers ranging from mono- to hexa-CBs to rats and found that 91-99% of the dose was retained in the body. After absorption PCBs are distributed throughout the body: their distribution tends to parallel the lipid content of the tissues, so that PCB storage in the tissues occurs in the following order:

fat > liver > feces > kidney > brain > plasma > urine (346).

The concentration of PCBs in adipose tissue is 10-100 times the concentration found in other tissues, both early after single doses (182) and after prolonged intake (346).

Allen *et al*. (196) fed six adult female rhesus monkeys 25 ppm of Aroclor 1248 for two months. PCB concentrations in samples of adipose tissue averaged 127 µg/g fat for all animals. At that time, the experimental diet was discontinued. Eight months later the PCB content was 34 µg/g fat. PCBs were transferred across the placenta of one female which gave birth and concentrated at high levels in the fat and adrenals of the infant (Table IV.2.12). In an infant born to a female 29 months following the discontinuation of PCBs, the levels within the adipose tissue were 3.38 ppm at 4 months of age (69).

Female monkeys given 5.0 ppm PCB in their diets attained maximum levels of PCBs within their adipose tissue at 6 months (141 to 177 ppm adipose tissue). However, it required approximately 14 months on the 2.5 ppm diet for the monkeys to reach similar maximum PCB levels in their adipose tissue (126 to 144 ppm). Males which received 5.0 ppm PCBs attained levels ranging from 128 to 200 µg per g adipose tissue at 14 months. Infants born of mothers exposed to 2.5 and 5.0 ppm PCBs within the diet contained concentrations of PCBs ranging from 1.0 to 4.8 ppm within the skin at birth. While nursing from mothers consuming PCB diets, the infants continued to accumulate the compound. At 3 months the levels within the tissues ranged from 86 to 136 ppm. The concentration of PCBs within the milk ranged from 0.15 to 0.40 ppm. The tissues of the infants which died while nursing PCB-fed mothers contained high levels of PCBs within the thymus, ovaries, brain, kidneys, adrenal glands and pancreas (20-48 ppm tissue). Lower levels were found in the liver, lymph nodes and bone marrow (8-16 ppm) (69).

Table IV.2.12
(from ref. 196)

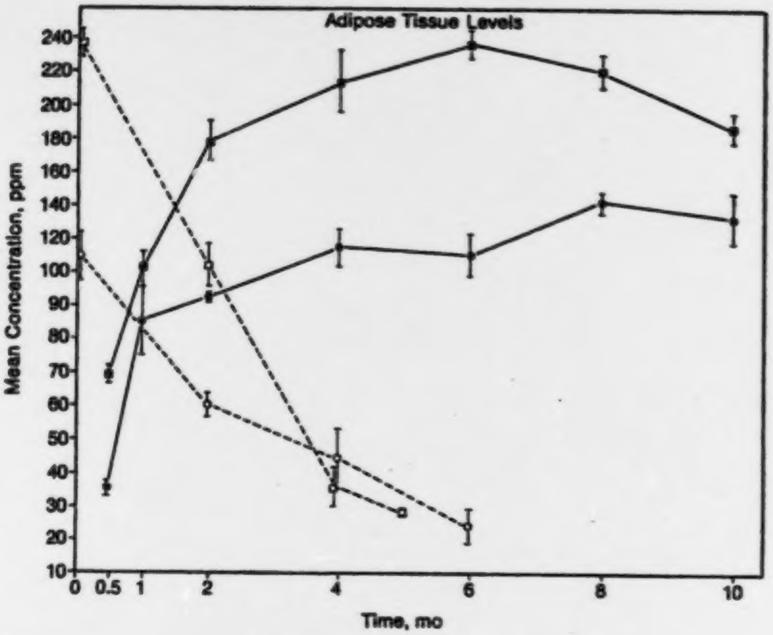
POLYCHLORINATED BIPHENYL CONTENT OF TISSUES OBTAINED FROM MOTHER AND INFANT FOLLOWING CONSUMPTION OF AROCLOR 1248 BY THE MOTHER PRIOR TO PREGNANCY

Organ	Weight (g)	PCB content (μg/g)
Mother		
Liver	—	56.3
Fat	—	50.0
Placenta	—	0.9
Infant		
Fat	—	27.70
Adrenals	0.2	24.40
Liver	11.9	0.01
Muscle	—	0.98
Brain	52.9	0.29
Kidneys	2.8	0.10
Large intestine	—	0.08
Small intestine	—	0.52
Stomach	—	0.55
Lung	6.1	0.21
Skin	—	0.31

Curley et al. (346) fed weanling Sherman rats a diet containing 100 ppm Aroclor 1254 for 58 days and for 240 days. Animals were sacrificed at various intervals. A steady buildup of PCB in all tissues was observed over the 58 day period. The rats stored more PCB in their tissues after 240 days than at the end of 58 days. In experiments at two dose levels (100 ppm and 500 ppm) for 240 days, Curley et al. (346) observed that PCB in fat after 240 days at dietary levels of 100 and 500 ppm were 1,101 and 10,021 ppm, respectively. They did not reach a point of equilibrium storage.

Grant et al. (347) fed male rats a diet containing either 0, 2, 20 or 100 ppm of Aroclor 1254 for 246 days. The residues in the blood, brain, heart, liver and fat were found to be dose related. All components of residues were not metabolized at the same rate.

Burse et al. (208) fed rats diets containing 100 ppm of Aroclor 1242 or Aroclor 1016 for up to ten months. Rats were sacrificed at intervals and tissues analyzed. A roughly steady state concentration of PCBs in adipose tissue was approached in two months and reached in 4-8 months (115 ppm of Aroclor 1242 and 214 ppm of Aroclor 1016) (Figure IV.2.12). Equilibrium levels of Aroclor 1016 and 1242 seem to be reached sooner than equilibrium levels of Aroclor 1254 and the levels of Aroclors 1016 and 1242 were 5-10 times smaller than those reached after continuous feeding with Aroclor 1254 (208, 346). This indicates considerably less bio-magnification of tri- and tetra-CBs than of penta- and hexa-CBs in rats. After the rats were placed on a PCB-free diet the residues of Aroclor 1016 were eliminated somewhat faster than those of Aroclor 1242 (Figure IV.2.12). Comparison



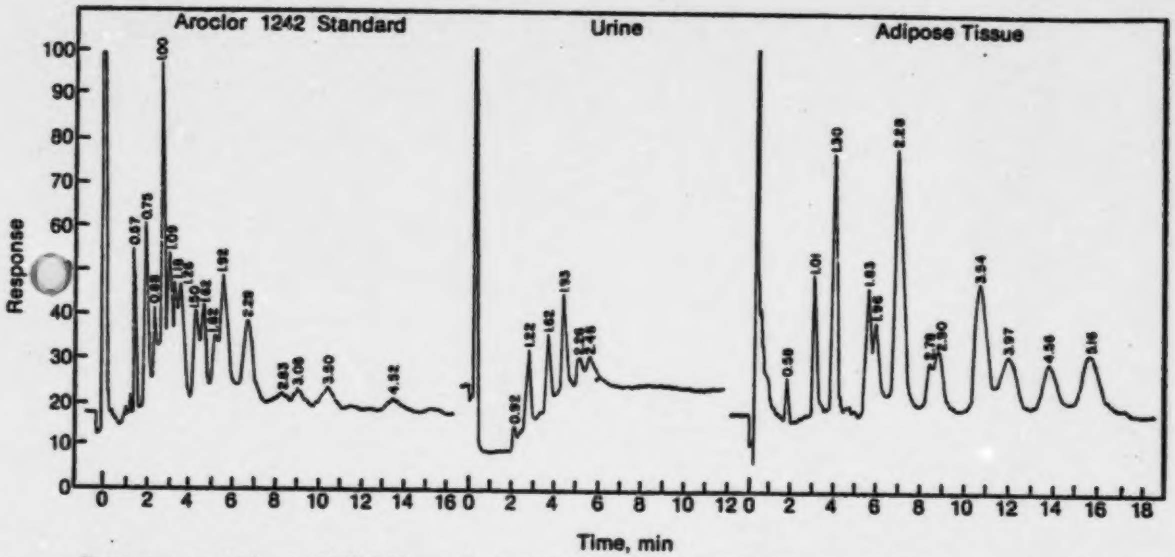
-Residues resulting from a continuous diet of a 100 ppm concentration of Aroclor 1016 (▪) or Aroclor 1242 (·) for a period of ten months. Residues following a continuous diet for only six months and recovery for five months for Aroclor 1016 (□) and for six months for Aroclor 1242 (o) are illustrated by the broken lines. Each point represents the mean and standard error (I) of four rats.

Figure IV.2.12
(from ref. 208)

of chromatograms (Figures IV.2.13, IV.2.14) shows that only the later eluting components (penta-, tetra-, and some tri-CBs) of Aroclors 1242 and 1016 were stored in the adipose tissue. The lower components were largely metabolized, as hydroxylated metabolites were found in the urine (348).

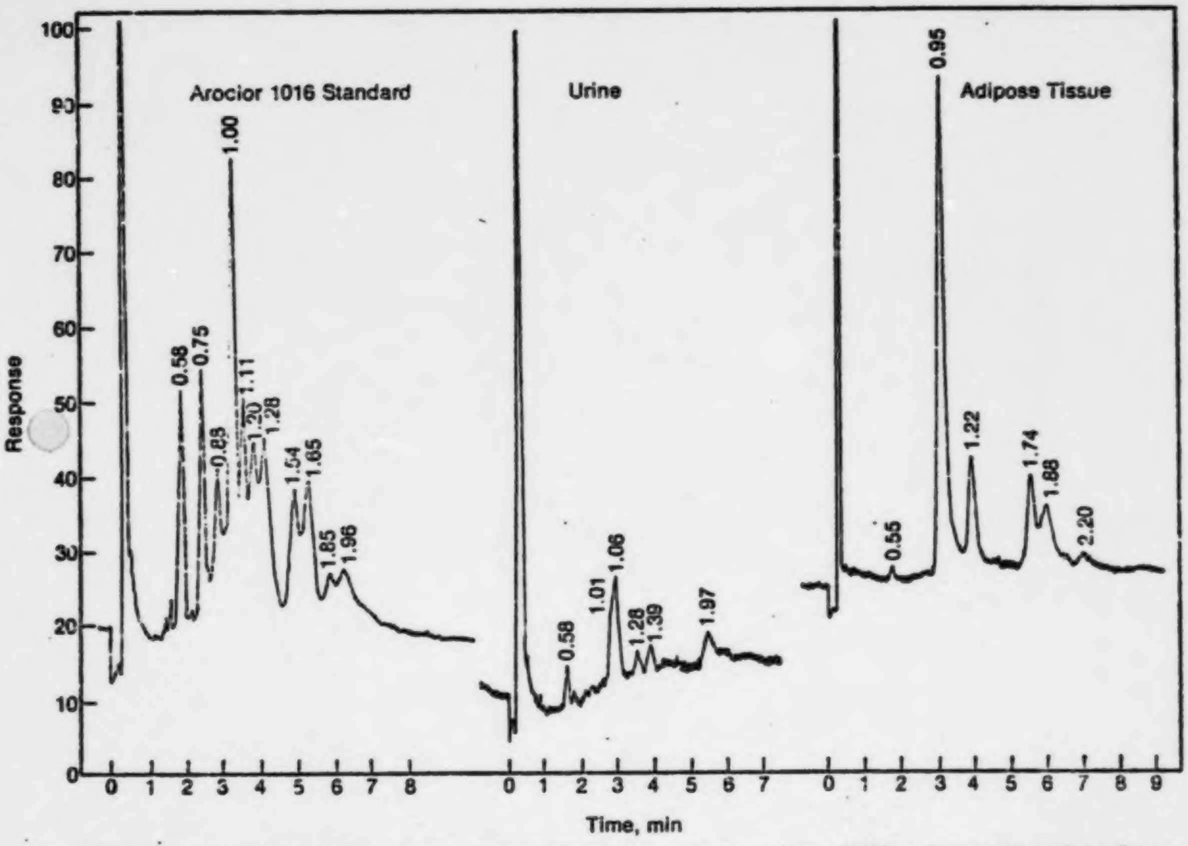
Storage and dynamics of PCBs have also been studied in domestic animals. Hansen et al. (216) fed sows with 20 ppm Aroclor 1242 throughout gestation and nursing and found PCBs in many tissues of the sows and their offspring. PCB levels in the fat and blood of the sows averaged 14 ppm and 0.25 ppm respectively (Table III.9.9). The offspring appeared to have somewhat lower concentrations of PCBs in their fat but higher concentrations in the blood (Figure IV.2.15). As with other mammals, there was considerable differentiation of the components of the Aroclor 1242 mixture (Figure IV.2.15): later eluting components (primarily penta- and tetra-CBs) were retained much better in the fat than the earlier eluting components. One-third to one-half of the PCBs stored in the fat of the sows consisted of components that would not be present in Aroclor 1016.

Fries et al. (349) fed 200 mg of Aroclor 1254 daily (0.4 mg/kg/day, equivalent to 12 ppm in the diet) to nine cows. Residues in the body fat and milk fat built up to 40 and 60 ppm respectively during a 60-day feeding period, but did not reach equilibrium levels in that time (Figure IV.2.16). Following discontinuation of dosing, levels of PCBs in fat and milk fell off steadily, but those in body fat had fallen only to 30 ppm after 60 days. The cows excreted only



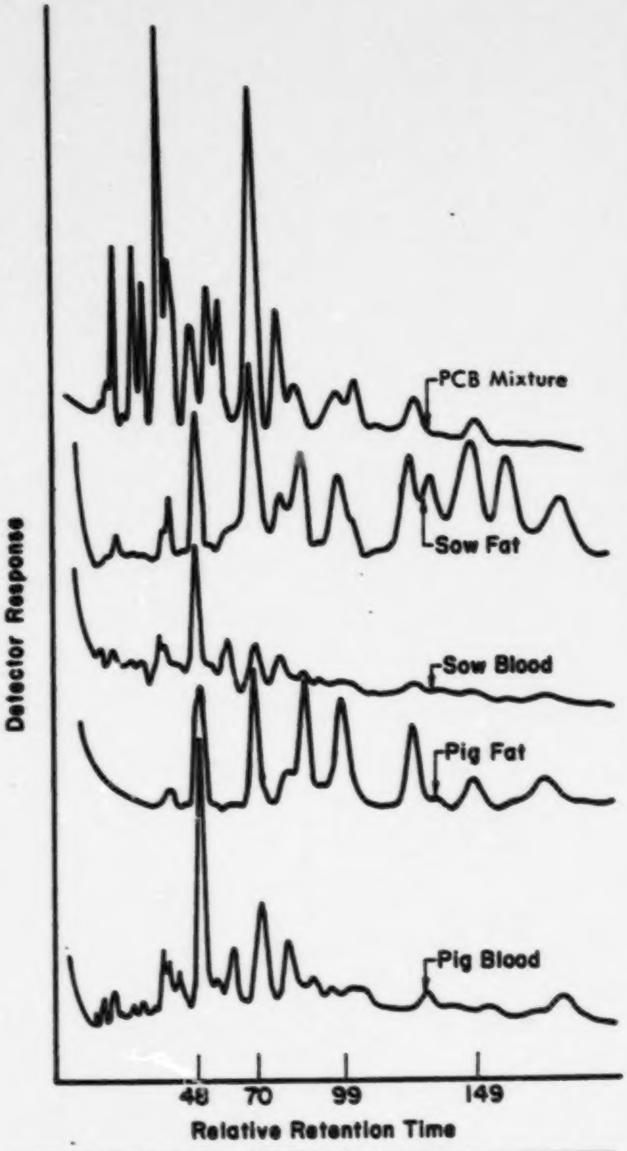
–Chromatograms of Aroclor 1242: 3.66 ng, an extract of urine: 4.5 mg, and adipose tissue: 0.027 mg from a male rat fed a diet containing 100 ppm Aroclor 1242 for eight months.

Figure IV.2.13
(from ref. 208)



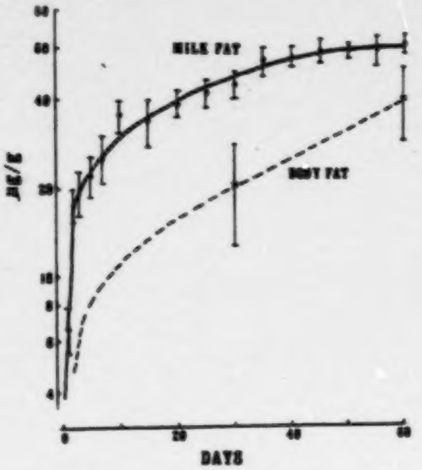
.—Chromatograms of Aroclor 1016: 2.84 ng, extract of urine: 5.4 mg, and adipose tissue: 0.008 mg from a male rat fed a diet containing 100 ppm Aroclor 1016 for eight months.

Figure IV.2.14
(from ref. 208)



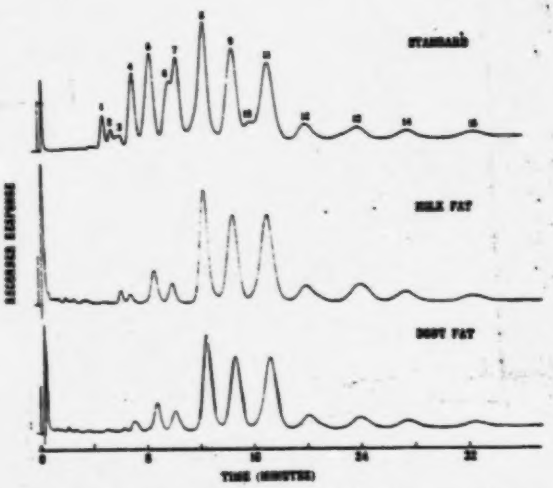
—Electron capture gas chromatographs of PCB mixture in standard and tissue extracts from sows given PCB mixture in the feed and from their offspring. Back-fat samples were diluted 100-fold.

Figure IV.2.15
(from ref. 208)



Concentration of PCB in milk fat and body fat of cows fed 200 mg of PCB per day. Each point is an average of nine cows ± standard deviation. The curve for body fat is extrapolated before 30 days.

Figure IV.2.16 (from ref. 349)



Typical gas chromatograms of an Aroclor 1254 standard, a milk fat residue sample, and a body fat residue sample. Retention times of peaks 1, 2, 4, 6, and 9 were equal to the retention times of the pure compounds 2,5,2′,5′-tetrachlorobiphenyl, 2,3,2′,5′-tetrachlorobiphenyl, 2,5,3′,4′-tetrachlorobiphenyl, 2,3,4,2′,5′-pentachlorobiphenyl, and 2,4,5,2′,4′,5′-hexachlorobiphenyl, respectively.

Figure IV.2.17 (from ref. 349)

20-25% of the daily dose in their milk, implying that even under conditions of continuous milk production they were still far from equilibrium after 60 days. There was differential accumulation of the higher components of the mixture in both body fat and milk (Figure IV.2.17), but peaks 2 and 3 (both tetra-CBs) were as well represented in the milk fat as in the original Aroclor mixture. Jan et al. (404) found that cows excreted substantial amounts of 3,5,3',5'-tetraCB in milk (up to 2 ppm in milk fat after a single oral dose of 0.8 mg/kg).

Frank et al. (405) reported data on body burdens of PCBs in captive harp seals (Pagophilus groenlandicus), as shown in the following table:

Age at Death and time in Captivity (months)	Wt. at Death (kg)	Estimate PCB Intake last 2-4 Months (mg/day)	PCB (mg)	PCB (mg/kg)
12-11	52.0	0.75	163	3.13
15-14	68.0	1.14	143	2.15
24-23	70.0	1.07	613	8.75
27-26	63.8	1.01	659	10.32
38-35	65.0	0.75	785	12.10
52-50	54.0	0.69	697	12.90

The diet of the seals contained an average concentration of about 0.2-0.3 ppm PCBs, but over 4 years' captivity the average whole-body concentration built up to 12.9 ppm (~~roughly 50~~ 25-38 ppm in fat).

IV.2.7. Storage and Bio-magnification in Humans

Although extensive surveys have been made of the occurrence of PCBs in human tissues, there is little systematic information on rates of uptake or elimination. PCBs are efficiently absorbed through the gastrointestinal tract: Price _et al_. (350) measured PCBs in diets, urine and feces of eight preadolescent girls in Virginia, and estimated absorption of the ingested PCBs as 88%. Humans are also exposed via air, water, and dermal contact, but it is generally believed that the major exposure is via the diet (1-3, 331, 351).

The most extensive data on distribution of PCBs in human tissues derive from Japan (35, 25). Fujiwara cited mean levels of 3.1 ppb in blood plasma, 4.7 ppm in body fat, and 50 ppb in milk of persons surveyed in the Kyoto area (25). He also cited a national survey which showed a mean level of about 34 ppb in 1116 samples of human milk. Kuratsune (35) cited a national survey which reported PCB levels in the range 0.04-1.7 ppm in skin, 0.3-6.4 ppm in fat, and 0.01 to 0.6 in liver (Table III.16.9). Although these figures do not all refer to the same individuals, they suggest a distribution in the body tissues paralleling that of lipids, as in other animals.

Surveys in Canada show that the majority of Canadians have residues of 1-2 ppm in their adipose tissue. Human milk of Ontario residents was found to have average residues of about 1.1 ppm PCBs on a fat basis (342).

Extensive surveys in the United States have shown that the median level of PCBs in human adipose tissue is slightly less than 1 ppm (Table IV.2.13) (353). Less information is available on the occurrence of PCBs in other human tissues. Finklea _et al_. (354) found mean levels of 2-3 ppb in blood plasma in South Carolina. Measurements of residues in human milk have ranged from not detectable up to 100 ppb; 30 ppb has been suggested as a typical level (331).

The Total Diet Survey of the Food and Drug Administration provides an estimate of the dietary intake of an average person in the United States: in recent years this estimate has been close to 9 µg/person/day (351: Table IV.2.14). The Total Diet Survey is based upon a high consumption diet which includes 4 kg food/day, almost twice that consumed by the "average" man (355). Hence the average concentration of PCBs in the food sampled in the Total Diet Survey is about 2.2 ppb. Comparing this with the median level in adipose tissue from the Human Monitoring Program (353), the storage factor for human fat (ppm in fat/ppm in diet) appears to be at least 400. This is far higher than that recorded for any experimental animal: see Section IV.2.6, where high figures for storage factors in mammalian fat range from 120-180 in seals, through 35-60 in monkeys, 11-20 in rats, and 5 in cows, to 0.7 in pigs. Several explanations are possible for this discrepancy.

Table IV.2.13 (from ref. 353)

Levels of polychlorinated biphenyls in human adipose tissue

Data source	Sample size	Percent nondetected	Percent < 1 ppm	Percent 1-2 ppm	Percent > 2 ppm
Yobs, 1972	688	34.2	33.3	27.3	5.2
FY 1973 Survey	1277	24.5	40.2	29.6	5.5
FY 1974 Survey	1047	9.1	50.6	35.4	4.9

Table IV.2.14 (from ref. 351)

Estimates of daily PCB intakes (total diet study--teenage male)

	Average daily intake of PCB's[a]	
Fiscal year	Total diet (μg/day)	Meat-fish-poultry food class (μg/day)
1971	15.0	9.5
1972	12.6	9.1
1973	13.1	8.7
1974	8.8	8.8
1975 (1st half)	8.7	8.7

[a] Lower limit of quantitative reporting = 0.05 ppm with analytical method employed.

(i) the dietary intake estimated by the Total Diet Survey may be too low, because of a high frequency of residues near the level of detectability;

(ii) routes other than the diet may give significant exposures;

(iii) the residues stored in fat may reflect much higher exposures in the past;

or (iv) humans may store PCBs much more efficiently than other animals.

Jelinek and Corneliussen considered (i) and attempted to minimize errors by assigning finite residue values to "trace" findings (351). Although other routes may be locally significant, no quantitative data have been provided that suggest intakes greater than a few micrograms per day for a typical person (2, 8, 331). The rapid decline of PCBs observed in the Yusho victims (Table III.16.9) makes it unlikely that present storage levels reflect exposures more than a few years ago. Hence it seems probable that humans do in fact store PCBs more efficiently than other animals. Humans are known to store other chlorinated hydrocarbons, such as dieldrin (335, 356) much more efficiently than other animals. This is of considerable importance for establishing "safe" levels for human intake, because it implies that for a given dietary concentration, humans will have tissue levels up to 10 or more times higher than those of experimental animals.

There is no precise information on the molecular or isomeric constitution of the PCBs stored in human adipose tissue in the

United States, although Kutz and Strassman state that most consist of penta-, hexa- and hepta-CBs (353). A detailed study of a pooled sample of human adipose tissue from Sweden indicated that it contained 4-6% tetra-CBs, 23-25% penta-CBs, 44-46% hexa-CBs, 21-22% hepta-CBs, 4% octa-CBs, and 0.6% deca-CB (Table II.4.1). Musial et al. (357) reported that most samples of human milk analyzed in Canada had PCBs similar in constitution to Aroclor 1254, but that peaks corresponding to Aroclor 1242 (i.e. tetra-CBs) were detected in all samples. Thus it seems likely that tetra-CBs, although minor, are not negligible constituents of the PCBs in human tissues.

IV.2.8. Bio-accumulation and Bio-magnification in Natural Ecosystems

Several writers have commented that bio-accumulation and bio-magnification factors observed in wild animals in the field are often much greater than those measured under controlled conditions in the laboratory. Some examples of this phenomenon as it relates to PCBs are given below.

Escambia Bay, Florida. Hansen (74) and Nimmo (330) have pointed out that bio-accumulation factors estimated for several wild species in Escambia Bay are much greater than those measured in the laboratory (99, 105; see Table IV.2.15). The discrepancies are especially great for crabs (> 230,000 in the bay, versus a maximum of 4,600 in the laboratory) and fish (> 670,000 in the bay, versus a maximum of 37,000 in the laboratory).

Table IV.2.15 (from ref. 330)

BIOACCUMULATION OF AROCLOR 1254 IN ESTUARINE ORGANISMS

	CONCENTRATION FACTORS	
ORGANISM	LABORATORY	ESCAMBIÁ BAY
OYSTER	165,000	> 100,000
SHRIMP	26,000	> 470,000
CRABS	4,600	> 230,000
FISHES	37,000	> 670,000

Hudson River, New York. In a field experiment, Veith *et al*. (358) held a number of fish of five species in a live-car (in contact with river water but isolated from sediments) for 14 days in the Hudson River below Fort Edward, N.Y. Whole-body residues of Aroclor 1016 increased to 1.8-3.8 ppm during the 14-day period of exposure. Veith *et al*. calculated the average concentration of Aroclor 1016 in the river to be 100-170 ppt, and hence estimated bio-accumulation factors in their test fish to be 10-40,000, comparable to those observed in the laboratory (358). However: (a) PCB levels in wild fish in the Hudson River have been up to 100 or more times higher than those observed in the test fish (Appendix C, 359); (b) Veith *et al*. calculated the PCB concentration in the river from the known discharges and the river flow: adsorption to the sediments is likely to have reduced the actual concentration considerably.

The Great Lakes. Extensive data are available on PCBs in water, sediments, and biota in the Great Lakes (Appendix C, pp. 31-54; 93, 360-368). Estimates of the degree of bio-accumulation are hampered by the difficulty of measuring concentrations of PCBs in water in the low parts per trillion characteristic of the open water of the lakes. The best set of data obtained by modern sampling techniques (use of polyurethane foam plugs for extraction from 20 liter samples -- see ref. 56) is from Lake Superior, where the mean PCB level at Duluth was estimated at 0.8 parts per trillion (363). Corresponding mean levels in biota were estimated as 0.1 ppm in zooplankton, 0.25 ppm in sculpins, and 0.3-3.3 ppm in several other fish species (363).

One of the fish species with the highest residues in this survey was the lake trout (Salvelinus namaycush), with mean residues of 0.7 to 1.9 ppm at various sampling stations in western Lake Superior (363). Another survey of lake trout in western and northern Lake Superior reported residues in the range 2.7-13.8 ppm (mean 7.0 ppm) in the whole fish, and 7.6-62.8 ppm (mean 25.6 ppm) in the fat (extracted oil) (367). Canadian data (360) fall into the same ranges, with the lake trout showing generally the highest residue levels (Table IV.2. 16). These data indicate bio-accumulation factors in fish in the range 3 x 10^5 to 9 x 10^6 (3 x 10^7 when expressed on a fat basis).

In Lake Michigan the average concentration of PCBs in water has not been measured precisely but appears to be substantially less than 10 ppt (362, 368): levels measured in the intake water of municipalities along the Michigan shore were all, with one exception, below 10 ppt (368), and even the concentrations reported in major tributary rivers to the lake have been only in the range 10-65 ppt (Appendix C, p. 46). PCB levels measured in fish in Lake Michigan have been consistently in the range 2-20 ppm, wet weight (16-200 ppm, lipid weight) (361, 368; Appendix C, pp. 40-45). These figures indicate bio-accumulation factors ranging up to at least 2 x 10^6 in whole fish (2 x 10^7 in lipids).

In Lake Ontario concentrations of PCBs in water in the range 35-56 ppt were reported in 1972 (Appendix C, pp. 31-32), but the method of extraction and analysis was not stated and the reportedly high levels in water are only partly reflected in high levels in

Table IV.2.16 (from ref. 360)

Polychlorinated biphenyls in commercial fish and fishery products, (area 3)

Location	Major species	Landings[a] (pounds)	PCB (mean ppm)
Lake Superior (A)	chub	306,000	0.96 (8)
	lake herring	1,611,000	1.17 (10)
	lake trout	195,000	2.02 (37)
	whitefish	328,000	0.68 (11)
	smelt	788,000	0.35 (6)
	yellow perch	67,000	0.31 (5)
		3,295,000	
Lake Huron (B)	chub	671,000	2.09 (17)
	whitefish	950,000	0.95 (8)
	carp	56,000	1.55 (4)
	suckers	176,000	1.33 (12)
	yellow pickerel	269,000	0.61 (9)
	sheepshead (drum)	29,000	0.75 (2)
	coho salmon	noncommercial	5.11 (6)
		2,151,000	
Lake Erie	alewife	332,000	1.22 (14)
	rock bass	49,000	0.27 (2)
	carp	41,000	1.27 (7)
	yellow perch	12,190,000	0.19 (10)
	smelt	15,760,000	0.42 (21)
	yellow pickerel	234,000	1.16 (9)
	white bass	2,346,000	1.26 (28)
	catfish	88,000	2.04 (8)
	bullhead	109,000	0.26 (4)
	sheepshead (drum)	354,000	0.74 (15)
	coho salmon	noncommercial	3.14 (8)
		31,503,000	
Lake Ontario (D)	bullhead	248,000	0.73 (12)
	yellow perch	699,000	1.23 (10)
	smelt	103,000	4.16 (17)
	white perch	290,000	1.84 (22)
	sunfish	203,000	0.74 (6)
	carp	395,000	1.69 (10)
	rock bass	42,000	1.76 (18)
	eel	222,000	17.14 (49)
	coho salmon	noncommercial	4.97 (3)
		2,205,000	

[a] Landings less than 25,000 pounds per species are not included.

biota. Average PCB levels in fish are similar or slightly higher than those in Lake Michigan, in the range 4-30 ppm (360). Taking the water data at face value, these indicate bio-accumulation factors in the range 10^5 -10^6, somewhat lower than those in the same fish in Lakes Michigan and Superior.

Data on eggs of fish-eating birds from the Great Lakes indicate further bio-magnification of PCB residues by the birds. Table IV.2.17 summarizes PCB residues in eggs of Herring Gulls (Larus argentatus) from the Great Lakes (366). The geographical pattern of contamination generally parallels that found in the fish (Table IV.2.17). Taking Lake Superior as an example, the mean PCB level of 60 ppm in gull eggs (roughly 1000 ppm on a lipid basis) is much greater than that of 0.3-7 ppm in fish (5-40 ppm in lipids) (363). This suggests a degree of bio-magnification by the birds much greater than anything reported in the laboratory (Section IV.2.5).

The California Current. Young et al. (12, 369-370) have presented extensive data on PCB residues in the marine environment off southern California. Residues in inshore areas are variable because of localized sources, and hence are difficult to use to derive field estimates of bio-accumulation. However, the maximum water concentration measured at the surface above one sewage outfall was 4 ppt (370, p. 332); corresponding tissue levels in fish, crabs, and mussels ranged up to 4.3, 3.8 and 1.7 ppm respectively, indicating bio-accumulation factors of the order of 10^6.

Young et al. also presented measurements of the background concentrations of PCBs in the California Current which flows into

Table IV.2.17 (from ref. 366)

Organochlorine residue levels[a] in Herring Gull eggs in the Great Lakes[b]

	n	DDE	DDD	DDT	Dieldrin	Heptachlor Epoxide	Mirex	Hexachloro-benzene	PCB[c]	Hg
Lake Ontario	39	22.6 (8.8-35.1)	0.09 (trace-0.83)	0.09 (0.02-1.04)	0.37 (0.08-1.08)	0.12 (0.01-0.36)	5.06 (1.95-18.6)	0.19 (0.01-0.72)	142 (73.8-261)	0.51 (0.29-1.47)
Lake Erie	42	7.04 (3.8-14.3)	0.08 (trace-0.24)	0.04 (0.01-0.15)	0.30 (0.10-0.69)	0.14 (0.04-0.28)	0.31 (0.14-2.19)	0.11 (0.06-0.31)	65.8 (41.2-110)	0.22 (0.11-0.35)
Lake Huron	40	13.8 (5.4-41.9)	0.10 (trace-0.38)	0.08 (0.01-0.32)	0.41 (0.13-0.87)	0.12 (0.04-0.26)	0.56 (0.06-6.92)	0.14 (0.05-0.42)	51.5 (15.4-118)	0.23 (0.11-0.50
Lake Superior	39	18.6 (8.6-47.1)	0.15 (trace-0.4)	0.12 (0.02-0.58)	0.39 (0.13-1.35)	0.14 (0.07-0.38)	0.66 (0.2-5.17)	0.11 (0.02-0.33)	60 (33.4-148)	0.39 (0.16-0.63)
Lake Michigan	10	31.8 (15.8-145)	0.01 (0.01-0.07)	0.13 (0.07-0.39)	0.48 (0.3-0.92)	0.16 (0.11-0.60)	<0.01	0.04 (0.02-0.14)	91.3 (55.1-395)	n.d.

[a] Median Values and ranges (in brackets) in parts per million, wet weight.

[b] Eggs were collected from two colonies in each lake in both 1974 and 1975 except from Lake Michigan where they were from a single colony in 1975.

[c] Polychlorinated biphenyl values are based on a 1:1 mixture of Aroclor 1260:1254.

n.d. Not detected.

the area from the northwest: these fell into the range 0.27-0.49 ppt with a mean of 0.40 ppt (369, p. 29). Away from the influence of local sources of PCBs, mean residues in mussels and dover sole were in the ranges 14-26 ppb and 40-50 ppb respectively. Referred to the reported background level in the water these indicate bio-accumulation factors of the order of 5 x 10^4 and 10^5 respectively. However, Risebrough _et_ _al_. (56, pp. 12-20) have presented evidence that the PCB levels reported in water by Young _et_ _al_. are at least 10 times too high; if so, these estimates of bio-accumulation factors would be at least 10 times too low.

Reported levels in birds and mammals in the California current and the outer islands are much higher than those reported in mussels and fish: 0.15-2 ppm in whole bodies of various fish-eating birds (371); 10 ppm in the whole bodies (200 ppm in lipids) in plankton-eating birds (225, 371); 20-400 ppm in the egg-lipids of fish-eating birds (372, 373); 12-145 ppm in the fat and 0.5-9.7 ppm in the livers of sea-lions (374). These figures indicate a substantial degree of bio-magnification by the vertebrates and an overall storage ratio (ppm in fat/ppm in ocean water) exceeding 10^9 in some cases.

Despite the variability in residue levels near to major sources of PCBs, the Southern California studies provide useful information about differential bio-accumulation of PCB components. At one of the largest sources, a sewage effluent at Palos Verdes, about 66% of the PCBs discharged during 1974-75 consisted of components characteristic of Aroclor 1242 (tetra- and tri-CBs): the remainder consisted of components characteristic of Aroclor 1254 (penta- and hexa-CBs) (369: Table 1). The corresponding ratio in the tissues of fish (dover sole) sampled at the same place was reversed (67% Aroclor 1254) (370: Table 3). This suggests that in these circumstances penta- and hexa-CBs were accumulated roughly 4 times more efficiently by fish than tetra- and tri-CBs.

The North Atlantic Ocean. Several authors have published measurements of the order of 1 ppt or higher for concentrations of

PCBs in seawater in the open Atlantic Ocean (52, 53, 375, 376), but the problems of sampling without shipboard contamination are very severe and Risebrough et al. (56) have given evidence that all the figures are much too high. Certainly 1 ppt is an upper limit for levels to be expected in ocean water.*

Discounting data from locally polluted inshore waters, PCB residues reported from biota samples from the open Atlantic Ocean include the following:

	PCB residues (ppm)		
Organism	Whole body or muscle	Lipid	Ref.
Zooplankton	0.01 - 0.12	0.1 - 9.8	378
"	0.1 - 0.45	0.3 - 260	379
Fish	0.002 - 2.1	0.4 - 31	376
"	0.008 - 0.1	0.2 - 3	380
"	0.07 - 2.65		360
Deep-sea fish		4 - 12.5	56
"	0.001 - 0.17	0.04 - 38	376
Oceanic birds	0.2 - 21	0.25 - 74	387
Seabird eggs (Iceland)	0.4 - 27	4 - 320	380
Seals		2 - 75	386

Despite the wide variability (and some problems in sampling plankton -- see ref. 56) these data indicate that PCB residues in plankton and fish often reach 0.1 ppm in the whole body and 1 or even 10 ppm in lipids. Even accepting the 1 ppt levels reported in seawater, these figures indicate bio-accumulation factors of the order of at least 10^5 (10^6- 10^7 in lipids). Plankton-eating and fish-eating birds and mammals often have residues an order of magnitude higher still. Levels were highest of all in predators (up to 88 ppm in muscle and 535 ppm in fat in Glaucous Gulls (Larus hyperboreus) at Bear Island -- ref. 381).

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* The first report of levels up to 150 ppt (375) has been questioned (377) and is inconsistent even with subsequent reports of around 1 ppt, which are themselves questionable.

The data cited above show that in natural environments animals generally bio-accumulate and bio-magnify PCBs by factors substantially higher than those usually measured in laboratory experiments. Despite the difficulties in measuring PCBs in water at levels near or below 1 ppt, there is now evidence from several areas that aquatic invertebrates and fish can bio-accumulate them by factors as high as several million fold from ambient water, and that fish-eating birds and mammals can further bio-magnify these concentrations by factors of 10-100. Reasons for this enhanced magnification in natural environments have been discussed by Nisbet (336). One likely reason is that PCB residues in many organisms increase with age (see Figure IV.2.18 for an example involving lake trout). Hence long-lived wild animals have an opportunity to accumulate PCBs to much higher levels than individuals exposed experimentally for limited periods in the laboratory.* Other reasons proposed for this field effect include selective exposure in a patchily contaminated environment, and selective predation on contaminated prey (336). Whatever the explanation for the phenomenon, the bio-accumulation factors observed in the field are those that are relevant to assessment of exposure to organisms that may eat the contaminated fish.

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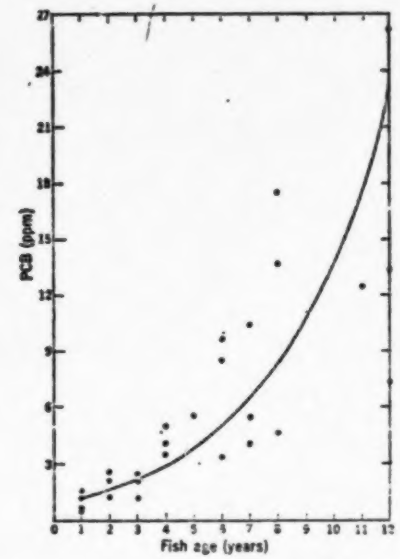
* See also ref. 405 for an example involving seals, whose body burdens were still increasing after 4 years in captivity (see p. 326 above).



The concentration of PCB's in Cayuga Lake trout as a function of age.

Figure IV.2.18 (from ref. 381)

IV.3. Presence in the Aquatic Environment

A review of PCB levels in the environment is appended to this document as Appendix C and only a brief summary is included here. PCBs are widely distributed in the environment of North America. Data gathered from nationwide monitoring activities by the U.S. Geological Survey (48) show mean concentrations of PCBs in the range 0.01 to 0.05 µg/l (10-50 ppt) in unfiltered water samples and from 0.25 - 218 ppb in bottom sediments. The highest levels were found in the Southeast (South Atlantic Slope and Eastern Gulf of Mexico drainage basins) and the Northeast (North Atlantic Slope and St. Lawrence River drainage basins). (See Appendix C, pp. 3-15).

In the National Pesticide Monitoring Program, PCBs have been detected in fish at all of the 100 monitoring stations, although the percentage of composites with detectable residues fell from 100% to 51% between 1969 and 1973 (Appendix C, p. 27). In the same period the number of composite samples of fish with residues greater than 5 ppm fell from 61% to 40% (*ibid*.). Generally, the higher concentrations were found in certain river systems with industrial activity, including the Allegheny, Kanasha, Cumberland, Tennessee, Ohio, Mississippi and Missouri Rivers (93). High residues have also been found in fish in the Great Lakes, in the Hudson and other northeastern rivers, and in a number of estuaries and inshore waters (Appendix C, pp. 31-87; 2, 93, 359-364, 367-370, etc.). PCB residues in fish detected in monitoring programs include chlorobiphenyls characteristic of Aroclors 1242 and 1248 (tri- and tetra-CBs) as well as those characteristic of Aroclors 1254 and 1260 (penta-, hexa-, and hepta-CBs (93, 370, 367, 382). For example, in the crosscheck samples analyzed for the National Pesticide Monitoring Program, 1970-73, 26 of 131 samples listed contained components characteristic of Aroclors 1232 or 1242, and 70 had components characteristic of Aroclor 1248; all had components characteristic of Aroclors 1254 and 1260 (382). In lake trout samples in Lake Superior, more than half the PCB residue components were listed as matching Aroclors 1242 and 1248 (367). Similarly, numerous tetrachlorobiphenyls and at least one trichlorobiphenyl component were identified in lake trout and coho salmon from Lake Michigan (95). Thus tri- and tetra-CBs as well as higher CBs are widely stored in fish in the United States.

PCBs are also found very widely and often at high levels in fish-eating birds (1, 2, 56, 93, 225, 364-366, 371-373) and fish-eating mammals (374, 383-386). Levels are particularly high in the fat (blubber) of marine mammals such as seals, dolphins and polar bears (383-386; see Table IV.3.1).

PCBs are widely distributed in human tissues, reflecting general intake in the diet (351-356).

Table IV.3.1 (from ref. 383)

TOTAL DDT PLUS PCBs IN BLUBBER OF HARBOR SEALS COLLECTED IN 1971

LOCATION	NO. OF SEALS	MONTH COLLECTED	AGE, YEARS	TOTAL DDT & PCBs (mg/kg, wet wt.) MEAN	INDIVIDUAL VALUES
Washington: Puget Sound	2	June-August	1	862.7	459.4; 1,620.0
California: San Miguel Island	5	June	9-30+	610.7	380.7; 318.5; 425.5; 518.3; 2,350.0
Oregon: Columbia River	3	May	2-4	62.5	27.7; 80.4; 109.9
Alaska: Pribilof Islands	3	August	(a.)	11.3	6.8; 7.1; 27.8

a. canine teeth lost

IV.4. Effects on Biota and Natural Ecosystems

Effects of PCBs on organisms and ecosystems fall into two categories: (a) effects observed in the laboratory under controlled conditions of exposure to PCBs; (b) effects observed in natural systems and associated with PCB exposure by epidemiological or equivalent methods.

Effects of type (a) are relatively easy to extrapolate to natural ecosystems: if an effect is observed in the laboratory when test organisms are exposed to a certain level of PCBs, it is reasonable to assume that similar effects will take place in the field if the same or comparable species are exposed to the same level of PCBs. It is often likely that effects will occur at lower levels in the field, for the following reasons:

(i) natural systems involve many species and it is likely that some will be more sensitive to PCBs than the species chosen for testing;

(ii) under natural conditions organisms are exposed intermittently to environmental stresses (temperature, pH, oxygen depletion, food shortage, predation, etc.) which generally make them more vulnerable to the effects of an additional toxic stress;

(iii) natural systems are exposed to other pollutants and it is known that additive and and synergistic toxic effects may occur.

Thus it is reasonable to expect adverse effects to occur in the field at lower exposure levels than in the laboratory, but it is difficult to predict the lowest hazardous levels without actual field studies.

Effects of type (b), however, which depend on field studies, are often difficult to interpret or to establish with reasonable certainty. When an adverse effect is observed in the field, it is often associated with exposure to more than one pollutant: it is difficult to establish whether it is caused by PCBs, by another pollutant, or by both acting together. Furthermore, PCBs are patchily distributed in the environment, so without extensive measurements of concentrations in water it is difficult to establish the exact degree of exposure of the affected organisms.

Accordingly to judge the effects of PCBs on natural systems it is necessary to weigh laboratory and field evidence together.

IV.4.1. Effects Observed in the Laboratory

The following effects of PCBs have been observed in controlled experiments at exposure levels which occur locally in the field:

(i) Change in species composition of phytoplankton communities (Section III.3). Effects on the growth and rate of cell division of sensitive species of phytoplankton have been noted at PCB concentrations as low as 0.1 ppb (84, 86) and large effects on the species composition of mixed cultures took place at this concentration (92). The effects of lower concentrations have not been studied. The changes involve replacement of sensitive diatoms by resistant green algae and are expected to have substantial effects on consumers and hence on food-webs (83). Synergistic effects with DDE are also involved (90).

(ii) Change in species composition of marine animal communities. Effects on species composition and biological diversity of communities of estuarine animals developing in the laboratory from planktonic larvae in flowing seawater were noted at PCB concentrations as low as 0.1 ppb, the lowest concentration tested (ref. 109; Section III.4.2).

The results reported in these experiments (refs. 88, 92, 109) are of particular importance because they demonstrate that PCBs affect mixed communities of plants and animals at concentrations much lower than those required to show effects on single species populations.

(iii) Effects on aquatic invertebrates (Section III.4). Reproduction and growth is impaired in aquatic invertebrates at concentrations lower than those required to cause mortality. The waterflea *Daphnia magna* and the midge *Tanytarsus dissimilis* are both affected at levels between 0.4 and 0.5 ppb (93: Table III.4.1). Synergistic effects with DDT are also involved (94).

(iv) Sublethal effects on fish (Section III.5). Although there is little evidence that PCBs affect survival of fish at concentrations below 1 ppb, adverse effects on reproduction have been noted at concentrations as low as 0.1 - 0.4 ppb, corresponding to residues of 5 ppm in eggs (113, 114, 115). Effects were also noted on thyroid function in fish exposed to a dietary level of 0.48 ppm PCBs (which corresponds to roughly 0.4 ppm whole-body residue) (refs. 73, 95; Figure III.5.2).

(v) Effects on reproduction in birds (Section III.6.4). Marked effects on reproductive success (especially embryonic mortality) have been noted in chickens at dietary levels as low as 8-10 ppm of PCBs, corresponding to residues of only 1-2 ppm in whole eggs. It is doubtful whether the chicken is a good model for wild birds, since other species have proved less sensitive in laboratory tests. However, second generation effects and synergism with DDE have been noted in other species at dietary levels of 10 ppm (161, 164).

(vi) Hepatic porphyria in birds (Section IV.6.3). PCBs induced ALA-synthetase and caused porphyria in quail at doses as low as 1 mg/kg/day and probably at 0.1 mg/kg/day. These effects were associated with residues as low as 1 ppm in the liver (145: Figure III.6.1).

(vii) Effects on mink (Section III.9.4). Mortality and total reproductive failure took place in mink fed a diet containing only 0.64 ppm of PCB residues, the lowest dose tested (201). In another experiment at a higher dose level, there was evidence of synergistic effects with DDT and dieldrin (200: Table III.7.5). The mink is the only fish-eating mammal whose sensitivity to PCBs has been tested.

All these effects have been noted at exposure levels which occur quite frequently in natural environments. Concentrations of 0.1 ppb are reported not infrequently from polluted fresh waters

(48; Appendix C), and concentrations as high as 0.4 ppb are reported occasionally. Fish throughout the United States have tissue residues as high as those associated with thyroid malfunctions (Appendix C; 93). Fish-eating birds often are exposed to fish with PCB residues as high as 8-10 ppm (Section IV.3); many, perhaps most, fish-eating birds in the United States have residues in their eggs as high as the 1-2 ppm that affect hatchability in chickens (Section IV.3.; 1, 2, 56, 225, 364-366, 371-373). Many fish-eating birds, even from remote parts of the Atlantic Ocean, have PCB residues in the liver as high as 1 ppm (140, 387, 388). Fish-eating mammals in the Great Lakes and many other areas of the United States will be exposed to diets containing 0.64 ppm of PCBs (Section IV.3; 93). Thus it is reasonable to suppose that at least some of the adverse effects reported in the laboratory are taking place in natural environments.

IV.4.2. Effects Observed in the Field.

Several adverse effects on wild animal populations have been reported which have been associated reasonably plausibly with exposure to PCBs.

(i) Effects on reproduction in salmon. Swedish investigators have reported a statistically significant association between hatching failure in eggs of Atlantic salmon and PCB residues. Concentrations in the range 0.4-1.9 ppm, wet weight (7.7-34 ppm, lipid weight) were associated with mortalities between 16 and 100 percent (118). This report was published only as a preliminary note and complete details are not available.

(ii) Reproductive failure in other fish. Several species of fish in Lake Michigan have not been reproducing effectively in recent years (409-410). Despite annual stocking of lake trout since 1965, for example, no wild-raised young have been collected (409). Eggs of Atlantic salmon and northern pike have shown very low hatchability in the laboratory (410). These reproductive failures have been associated circumstantially with residues of PCBs and other chemicals, but no clear link has yet been established.

(iii) Fin rot syndrome in marine fish. "Fin rot syndrome" has been induced in the laboratory in spot exposed to 3-5 µg/1 of Aroclor 1254 (106). The syndrome appears identical to that observed in wild fish (croakers and spot) which died under conditions of warm weather and oxygen depletion in Escambia Bay, Florida (106). Although the deaths of the wild fish were attributed originally to lack of oxygen in the water (99), the levels of PCBs in the dying fish (around 10 ppm) were similar to those in fish experimentally poisoned in the laboratory (99, 111). This suggests that PCBs may have played at least a contributory role in the incidents.

In independent observations, McDermott et al. (370) have reported that fin erosion has recently become prevalent in dover sole off Palos Verdes and Orange County, California. Diseased sole have higher PCB residues than healthy fish (median concentration 2 ppm, wet weight, versus 1 ppm), but the difference was not quite statistically significant ($P = 0.1$). The syndrome was associated with concentrations of PCBs in water not greater than 4 ppt (370, p. 32).

(iv) *Deaths of fish-eating birds*. Koeman (142) reported that a number of Cormorants (*Phalacrocorax carbo*) found dead in the Netherlands in 1970 contained high residues of PCBs (mean 190 ppm in brain, 320 ppm in liver, wet weight). These concentrations were similar to those in Cormorants experimentally poisoned with PCBs (76-180 ppm in brain, 210-290 ppm in liver) and Koeman attributed the deaths of the wild birds primarily to PCB poisoning (142). However, the corresponding levels of PCBs in the fish and water were not known.

In November 1969, more than 50,000 seabirds, mostly murres (*Uria aalge*), were found dead in the Irish Sea. The most consistent findings at post mortem were emaciation, liver damage, and high residues of PCBs in the liver (2-880 ppm, wet weight) (389). Birds collected in apparent good health at the same time and place had body burdens similar to those of the dead birds, but in the healthy birds the PCBs were stored in the fat, whereas in the dead birds the PCBs were largely in the liver. It therefore seems likely that food shortage triggered the incident, but since the liver residues of PCBs in some birds were well into the lethal range (142) it seems likely that some of the birds actually died from PCB poisoning. This is an important example of the interaction of environmental and toxic stresses. Residues in fish in the same area were in the range 0.01-2.6 ppm (389).

Glaucous Gulls (*Larus hyperboreus*) which had been feeding on the eggs of seabirds at Bear Island in the Arctic Ocean had high levels of PCBs in the liver (mean 24 ppm). One individual found dying with failure of coordination of the limbs had 311 ppm PCBs in the liver (388) and is therefore likely to have succumbed to PCB poisoning (140, 142). The example is important because the PCBs were apparently acquired through the natural food chains of the North Atlantic and Arctic Oceans (387, 388).

Two Bald Eagles (Haliaetus leucocephalus) have been reported dying with high residues of PCBs in the brain. One found dead in Michigan in 1970 had 235 ppm of PCBs and 385 ppm of DDE in the brain (390). Another found dead in Michigan in 1972 had 190 ppm of PCBs and 55 ppm of DDE in the brain and 1200 ppm of PCBs in the whole body: the immediate cause of death was drowning, as it was seen to fall off a perch into the water (391). In each case the residues of ~~both~~ PCBs in the brain were in the lethal range (142, 391) and in the first case residues of DDE were also: it seems likely that the birds died from the combined effects of PCBs and DDE poisoning. The first bird was from the Lake Michigan area (Grand Traverse county); the second bird was probably from southeastern Michigan. The Bald Eagle is an endangered species and is subject to the special provisions of the Endangered Species Act of 1973.

(v) Effects on reproduction in fish-eating birds. Herring Gulls are experiencing low reproductive success at colonies in Lake Ontario, but appear to be breeding normally at colonies in the other Great Lakes, including Lake Michigan (366, 392, 393). The breeding failure is characterized by early embryonic mortality (393) and is associated with high levels of PCBs in the eggs (Table IV.2.17).

Recent studies have shown that the embryonic mortality is associated with porphyria and edema (subcutaneous, pericardial, and abdominal), and that these conditions are statistically associated with high PCB levels (406). In addition, deformities of the feet, toes, and eyes have been recorded in chicks of Herring Gulls and three other fish-eating species in Lake Ontario (407).

In studies of other species of fish-eating birds, reproductive failure and eggshell-thinning have been associated primarily with residues of DDE (56, 364, 372, 373, 394) and no other study has suggested an independent effect of PCBs. However, only a few species have been studied to date.

(vi) Premature births in sea-lions. Premature pupping in sea-lions has been noted on San Miguel and San Nicholas Islands, California, since 1968. Most or all of the premature pups die (374). Residues of PCBs were much higher in the females giving birth to premature pups than in those of females giving birth at full term (means 112 ppm and 17.1 ppm PCBs in blubber, respectively). However, residues of DDT compounds, primarily DDE, were also elevated in the females giving birth prematurely (means 824 vs. 103 ppm in blubber). Fish in this area have residues generally in the range 0.01-2 ppm PCBs, ranging up to 4-6 ppm in the vicinity of sewage outfalls (370).

In all cases summarized above, the association between PCB levels and adverse effects in wild animals is somewhat circumstantial: this is inevitable in an epidemiological type of investigation. However, in each case the suspected adverse effect can be matched with adverse effects noted in laboratory animals with comparable residue levels. In several of the cases the adverse effects were also associated with high levels of DDE, and additive or synergistic effects seem likely. The interaction of environmental and toxic stresses is also apparent in several cases. Together these examples seem sufficient to show that current levels of environmental contamination with PCBs are having adverse effects on natural ecosystems, but the ambient concentrations at which they do so has not been established.

IV.5. Potential Effects in the Human Population.

As detailed in Section III, several different toxic effects have been observed in experimental animals exposed to PCBs in the diet at levels as low as 0.5-2.5 parts per million. Surveys by the U.S. Food and Drug Administration suggest that the average level of PCBs in the human diet in the United States is now about 2.2 parts per billion (Section IV.2.7 above). There is thus a factor of 200-1000 between the average level in the human diet and levels known to cause effects in experimental animals.

In addition, residues of PCBs are sporadically distributed in the food supply and some individuals ingest much more than the average: persons with dietary preference for fresh-water fish and breast-fed infants have been identified as high-risk groups (2, 331).

It is not yet possible to use epidemiological studies to estimate safe levels of intake, for the following reasons:

(i) Epidemiological studies in the general population are not being conducted, and in any case would be difficult or impossible because of the widespread exposure of the general public.

(ii) The Yusho incident, although providing some dose-response information, is confused by the high level of PCDFs in the ingested PCBs.

(iii) Occupational experience in the past has provided no quantitative dose-response information and in any case may be confused by effects of PCDFs in older formulations.

(iv) Current studies of occupationally exposed workers are still in progress and in any case will provide information only about adults.

V. Criteria Formulation

The criterion level for PCB's is 0.001 ug/l PCB's. It should be noted that if all PCB entry into the environment were to stop immediately, the environmental burden would in some waters still exceed the ambient water quality criterion. While EPA is attempting to prevent the continuing discharge of PCB's, or at least to minimize the continuing discharge of PCB's into the aquatic environment, the responsiblity for protecting man from contaminated interstate commercial food products is with the Food and Drug Administration of the Department of Health, Education, and Welfare.

The chronic effects of PCB's in man may occur at extremely low concentrations. Although it becomes virtually impossible to state with confidence that any PCB concentration above zero provides an ample margin of safety for man, the PCB criterion number is believed to provide protection for the aquatic environment.

The criterion for PCB's is derived with consideration of the bioaccumulation potential rather than solely upon the more traditional use of an application or safety factor applied to an acute toxic value. The reasons for this approach are the environmental persistence and bioaccumulation potential of PCB's as outlined below:

The persistence of PCB's in the environment is such that they present long-term environmental hazards.

The laboratory determination of the bioaccumulation potential for PCB's in general has been shown to be around 274,000 times the PCB level in the test water. There have been reports that certain animals captured from the aquatic environment have apparently bioaccumulated PCB's in their flesh to levels of between 3 and 10 million times that of the water concentration in which the animals were captured. It is important to note, however, that the level of PCB contamination in the food of the captured aquatic life, as well as the concentrations of PCB's in which these organisms resided for the predominance of their life periods, are unknown. Thus, the actual bioaccumulation potential of PCB's in the aquatic environment is uncertain. Therefore, the bioaccumulation potential demonstrated in the laboratory of 274,000 is the number which is used in determining a criterion level.

The demonstrated physiological effects of PCB's on fish and consumers of fish and wildlife, which include reproductive failures in a broad phylogenetic group of aquatic and terrestrial animals, liver enlargement, enzyme induction and skin lesions, require the consideration of parameters beyond the usual acute toxicity and safety factors.

Data which support a one part per trillion (0.001 ug/l) criterion are:

0.1 ug/l will cause population shifts in phytoplankton. It is important to note that at this level of the food web bioaccumulation does not play a role in the adverse effect. However, this concentration is extremely low and does have significant ecosystem consequences since shifts in population at this level will have an impact on the food for top predators.

0.4 to 0.5 ug/l lowers the reproductive potential of some invertebrates.

0.1 ug/l will lower the species diversity index of some invertebrates.

Bioaccumulation has been demonstrated in the laboratory at concentrations of around 274,000 times the water level.

PCB contaminated fish will cause detrimental effects on consumers of fish.

At 0.64 ppm (milligrams per kilogram in the food), total reproductive failure was observed in mink.

At 3.57 ppm (milligrams per kilogram in the food), mink died.

At 2.5 ppm (milligrams per kilogram in the food), reproductive dysfunctions occurred in the rhesus monkey.

At 3 ppm (milligrams per kilogram in the food), some mortality was observed in the rhesus monkey.

In rats, 5 ppm (milligrams per kilogram in the food) resulted, in the induction of enzymes in the liver.

At levels of between 40 and 300 ppm PCB's in chicken feed, deaths of chicken occurred. Reproductive failures occurred at between 8 and 10 ppm PCB's in the food.

Ulceration in the stomachs of dogs occurred after long-term feeding at 1 ppm PCB in feed.

Based upon the highest bioaccumulation factors demonstrated in the laboratory, a water criterion of no more than 0.001 ug/l (1 part per trillion) should provide protection to the aquatic ecosystem, and should provide a margin of safety to the consumers of aquatic life.

Adverse effects in man that are due to PCB or polychlorinated dibenzofurans (PCDF) contamination have included: chloroacene, swelling of the eyes, and liver involvement. There was no quantification of the levels of PCB's ingested by those individuals affected by PCB's. The carcinogenic threat due to PCB's has not been conclusively demonstrated, although based upon interpretation of data, some researchers have concluded that PCB's have resulted in cancer in the mouse and thus they represent a threat to man.

Based upon the proven bioaccumulation potential of 274,000 times the ambient water concentration in controlled conditions, the level of 0.001 ug/l PCB's should afford protection for consumers whose sole diet consists of aquatic organisms contaminated at the worst or maximal level predicted by the laboratory data.

EPA - 560/7-76-001

Appendix C

REVIEW OF PCB LEVELS IN THE ENVIRONMENT

JANUARY 1976

OFFICE OF TOXIC SUBSTANCES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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REVIEW OF PCB LEVELS IN THE ENVIRONMENT

Prepared by

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Washington, D.C. 20460

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1.0 Introduction

Polychlorinated biphenyls (PCB's) were first manufactured commercially in 1929. They are currently manufactured in the United States, Great Britain, France, Germany, USSR, Spain, Italy and Czechoslovakia. The sole producer of PCB's in the United States is the Monsanto Industrial Chemicals Company, where products are marketed under the trade name "Aroclor". Various Aroclor products that have been marketed include Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262 and 1268. The latter two digits designate the percent chlorine in each formulation. Aroclor 1016 is a new product which has 41% chlorine. Since their introduction, PCB's have been used in a variety of commercial and industrial products such as transformers, capacitors, paints, inks, paper, plastics, adhesives, sealants and hydraulic fluids. Through this widening product usage and their resistance to degradation, PCB's have been identified in the environment since 1966 with increasing frequency.

On September 1, 1971 an interdepartmental task force was established in the United States to review the data linking PCB's with adverse health and environmental effects and "to coordinate a governmentwide investigation into PCB contamination of food and other products". The task force concluded in its report of May 1972 that PCB's were highly persistent, could bioaccumulate to relatively high levels in fish and could have serious adverse effects on human health. It recommended the discontinuance of all PCB uses except in closed electrical systems. Since the 1972 Task Force Report, environmental sampling and laboratory studies have indicated that PCB's were a more serious and continuing environmental and health threat than had been originally realized. These concerns were highlighted this past November at the National Conference on Polychlorinated Biphenyls held in Chicago, Illinois.

This study reviews the current PCB data base to assess the PCB levels in the environment on a national level; the full spectrum of PCB levels reported in man and the environment were of interest. Data were obtained from a number of national monitoring programs, the literature and many unpublished reports. The data examined was inclusive to December 1, 1975.

It should be stressed at the outset, that due to the complexity and difficulty of PCB identification and measurement, that levels reported are not really comparable between different investigators. This aspect could not be compensated for or identified in the data presented. In addition, some of the Aroclor identifications reported may only have been best estimates of the pattern observed and may not have really identified the true Aroclor discharged or the quantitative level of the polychlorinated biphenyl found in the sample. However, the levels reported do give an indication of the contamination levels in different media for a given locality.

Recognizing the faults of combining different data bases, the sheer mass of data supports the conclusion that there is widespread contamination of the environment by PCB's. There are regional variations, but effects are consistent across all media (e.g., water, sediment, fish, birds), generally showing greater concentrations of PCB's in highly developed areas and in areas of industrial activity. Over the years examined, the situation has shown no improvement nationally.

This report is divided into five major sections:

PCB's in the environment

Behavior in the environment

Occurrence in food

Bioaccumulation in man

Environmental trends

Wherever possible, the level of data reported has been greatly reduced over that originally reported, especially for the older studies, however every effort has been made to indicate the locality and the level of contamination originally reported. More recent studies are presented in greater detail. Trend assessments, cross media comparisons and conclusions are drawn where justified.

2.0 PCB Levels in the Environment

2.1 Data from National Monitoring Programs

2.1.1 Water

PCB monitoring in water is an activity of the overall National Pesticides Monitoring Program operated by the U.S. Geological Survey. Analysis of the PCB data in EPA's STORET water quality file has provided little information, although scattered whole water measurements have been taken by a number of states over the years. Those states reporting nonzero readings are few in relation to the number of states showing zero concentrations.

At the direction of various local, state and federal agencies, the USGS collects and analyzes water data, producing a data file of uniform integrity. Current data from this file[8] covering the period October 1972 to 1975 provides the best available national data and the basis for the following analyses.

PCB levels, gathered by the USGS during the period January 1971 to June 1972 were reported by Crump-Wiesner, Feltz and Yates[2]. While the data suffers from the lack of representative sampling within states and multiple samples from the same locations, they concluded that significant concentrations of PCBs were widespread in the water resources of the nation. Their published summary of PCB residue data for surface and ground waters has been updated with the 1972-1975 data and is presented in Table 2.1-1.

The preponderance of zero readings in whole water and the low levels of scattered non-zero readings could be masking widespread PCB contamination in the nation's waters. Zero readings are due to the low solubility of PCBs and the analytical procedure commonly used which limits detection to 0.1 ppb.

There is a disparity between whole water and bottom deposit measurements. In almost all cases in which samples of both whole water and bottom sediment were taken simultaneously and the latter reading was non-zero, the whole water concentration was measured at zero ppb This occurred even when the concentration in bottom sediment was as high as 4000 ppb[8]. More recent water studies[3] to the ppt level have consistently shown measureable PCB levels.

Table 2.1-1
Summary of PCB Residue Data for Surface and Ground Waters, January 1971-(partial) 1975

State	Year	No. of Samples	Occurences	Concentration (ppb)	Median Concentration (ppb)
Alaska	71	3	0	——	——
	73	-	-	——	——
Alabama	73	4	0	——	——
	74	2	0	——	——
Arizona	71	8	0	——	——
	72	8	0	——	——
	73	38	0	——	——
	74	14	0	——	——
	75	4	0	——	——
Arkansas	71	32	0	——	——
	72	8	0	——	——
	73	28	1	0.2	——
	74	22	0	——	——
	75	1	0	——	——
California	71	161	2	0.1, 0.1	——
	72	27	0	——	——
	73	110	0	——	——
	74	99	2	0.1, 0.1	——
	75	42	0	——	——
Colorado	71	32	0	——	——
	72	3	0	——	——
	73	6	0	——	——
	74	9	0	——	——
Connecticut	71	13	6	0.1-0.2	0.1
	72	16	6	0.1	0.1
	73	45	1	0.1	——
Delaware	NO ACTIVITY				
D.C.	NO ACTIVITY				
Florida	72	16	0	——	——
	73	106	4	0.1-1.0	0.1
	74	60	0	——	——
	75	22	0	——	——

Table 2.1-1 (cont.)

State	Year	No. of Samples	Occurences	Concentration (ppb)	Median Concentration (ppb)
Georgia	73	1	0	——	——
	74	29	0	——	——
Hawaii	71	5	0	——	——
	72	1	0	——	——
	73	1	0	——	——
	74	1	0	——	——
Idaho	72	1	0	——	——
	73	1	0	——	——
	74	6	0	——	——
Illinois	74	1	0	——	——
	75	1	0	——	——
Indiana	74	1	0	——	——
Iowa	71	24	0	——	——
	72	1	0	——	——
	73	27	1	0.1	——
	74	2	0	——	——
Kansas	71	10	0	——	——
	72	4	0	——	——
	73	24	1	0.2	——
	74	3	0	——	——
Kentucky	71	7	0	——	——
Louisiana	71	9	0	——	——
	73	195	0	——	——
	74	147	0	——	——
	75	22	2	0.1-0.2	——
Maine	71	2	0	——	——
	72	1	0	——	——
	73	6	0	——	——
	74	1	0	——	——
Maryland	71	6	1	0.1	——
Massachusetts	71	5	1	0.2	——
	73	5	1	0.1	——
	74	6	1	0.1	——
	75	5	1	0.1	——

Table 2.1-1 (cont.)

State	Year	No. of Samples	Occurences	Concentration (ppb)	Median Concentration (ppb)
Michigan	71	2	0	—	—
	72	2	1	0.1	—
	73	6	0	—	—
	74	23	0	—	—
	75	18	0	—	—
Minnesota	71	3	2	0.1-0.3	—
	73	1	0	—	—
	74	1	0	—	—
Mississippi	71	8	0	—	—
	73	1	0	—	—
	74	52	0	—	—
	75	60	0	—	—
Missouri	71	21	0	—	—
	72	7	0	—	—
	73	19	0	—	—
Montana	71	47	0	—	—
	72	9	0	—	—
	73	32	0	—	—
	74	8	0	—	—
Nebraska	71	44	0	—	—
	72	3	0	—	—
	73	24	0	—	—
	74	17	0	—	—
	75	2	0	—	—
Nevada	72	4	0	—	—
	73	6	0	—	—
	74	5	0	—	—
New Hampshire	73	3	0	—	—
	74	3	0	—	—
New Jersey	71	11	3	0.1	0.1
	72	5	1	<0.5	—
	73	29	2	<0.1,<0.1	—
	74	29	0	—	—
New Mexico	71	36	0	—	—
	72	10	0	—	—
	73	5	0	—	—
	74	5	0	—	—

Table 2.1-1 (cont.)

State	Year	No. of Samples	Occurences	Concentration (ppb)	Median Concentration (ppb)
New York	71	325	52	0.1-4.0	0.3
	73	32	1	0.1	—
	74	16	0	—	—
North Carolina	72	3	0	—	—
	73	1	0	—	—
	74	1	0	—	—
North Dakota	71	40	0	—	—
	72	3	0	—	—
	73	4	0	—	—
	74	3	0	—	—
Ohio	72	14	2	0.1-E.2	—
	73	1	0	—	—
	74	3	0	—	—
Oklahoma	71	19	0	—	—
	72	5	0	—	—
	73	77	2	0.1-2.9	—
	74	37	5	0.1-0.2	0.1
	75	11	2	2.0-3.0	—
Oregon	71	13	0	—	—
	72	2	0	—	—
	73	9	0	—	—
	74	9	0	—	—
	75	1	0	—	—
Pennsylvania	71	2	0	—	—
	73	1	0	—	—
	74	25	0	—	—
	75	20	0	—	—
Rhode Island	73	3	0	—	—
South Carolina	72	2	0	—	—
	73	2	0	—	—
	74	12	0	—	—
South Dakota	71	18	0	—	—
	72	1	0	—	—
	73	5	0	—	—
	74	1	0	—	—
Tennessee	73	1	0	—	—
	74	2	0	—	—

Table 2.1-1 (cont.)

State	Year	No. of Samples	Occurences	Concentration (ppb)	Median Concentration (ppb)
Texas	71	660	12	0.1-3.0	0.4
	72	82	1	0.1	—
	73	385	6	0.1-0.6	0.3
	74	297	4	0.2-0.7	0.3
Utah	72	1	0	—	—
	73	8	0	—	—
	74	16	0	—	—
Vermont	73	1	0	—	—
Virginia	71	4	1	0.1	—
	72	12	0	—	—
	73	5	0	—	—
	74	1	0	—	—
Washington	71	25	0	—	—
	72	1	0	—	—
	73	6	0	—	—
	74	6	1	0.1	—
West Virginia	71	4	0	—	—
	74	1	0	—	—
Wisconsin	71	3	0	—	—
	72	1	0	—	—
	73	12	0	—	—
	74	30	0	—	—
	75	2	0	—	—
Wyoming	71	18	0	—	—
	72	1	0	—	—
	73	6	0	—	—
	74	3	0	—	—
Puerto Rico	73	34	0	—	—
	74	63	1	0.8	—

E - estimated.

SOURCE: This table is an extension of Table 2 in Crump-Wiesner, J.S., H.R. Feltz and M.L. Yates, J. Research USGS, 1, 603-607; (1972) incorporating the newer data of PCB Data Base, October 1972 to (partial) 1975, The U.S. Geological Survey, Quality of Water Branch, Reston, VA.

2.1.2 Sediment

Nationwide sampling of bottom sediments have shown PCB concentrations to have widespread occurrence. Data from Crump-Wiesner, et. al.,[2] January 1971-June 1972 are summarized in Table 2.1-2. The October 1972 to 1975 USGS data are summarized in Table 2.1-3. Table 2.1-3 permits distinguishing between those states where the non-zero PCB readings were from one or a limited number of stations with multiple readings and those states where the non-zero readings come from a number of stations within the state. The map in Figure 2.1-1 indicates the distribution of PCB contamination from 1972-1974 based on the figures in the tables.

States which monitored sediment concentrations to any appreciable extent detected the presence of PCB's in concentrations often higher than 40 ppb. A greater proportion of states are reporting PCB's in bottom sediment each year, as displayed in Table 2.1-4.

Although the sediment data is meaningful, some caution is necessary in interpretation: measurements may be the result of past contamination, not present discharges; mean readings and ranges are somewhat inadequate to summarize the data - geographic locations are essential since readings show patterns only in particular water basins.

Table 2.1-2
Summary of PCB Residue Data for Bottom Sediments
January 1971 - June 1972

State	No. of Samples	Occurrences	Concentration (ppb)	Median Concentration (ppb)
Alaska	3	0	——	——
Arkansas	23	4	20-2,400	60
California	13	3	20-190	85
Connecticut	1	1	40	——
Hawaii	4	0	——	——
Georgia	12	10	10-1,300	300
Maryland	11	5	10-1,200	30
Mississippi	8	2	50;170	——
New Jersey	12	10	8-250	20
Oregon	4	2	15;140	——
Pennsylvania	16	11	10-50	20
South Carolina	11	8	30-200	50
Texas	293	23	7.9-290	80
Virginia	10	8	5-80	40
Washington	10	0	——	——
West Virginia	2	1	10	——

SOURCE: Crump-Wiesner, J.S., H.R. Feltz and M.L. Yates,
J. Research USGS, 1, 603-607, (1972).

Table 2.1-3
Summary of PCB Residue Data for Bottom Sediments
Stations measuring/#Stations with at least one non zero reading
Range of non-zero readings (mean), in ppb

STATE	1972	1973	1974	(partial) 1975
Alabama	—	1/0	2/0	—
Alaska	—	1/0	—	—
Arizona	1/0	1/0	1/0	—
Arkansas	6/0	6/0	15/4 <2-29 (12.8)	—
California	1/0	15/0	18/2 <2-65 (33.5)	—
Colorado	1/0	1/0	1/0	—
Connecticut	16/8 <5-E800 (127.5)	16/11 <5-40 (14.1)	40/31 <2-350 (71.5)	—
Delaware		NO ACTIVITY		
Florida	27/7 <5-400 (93.6)	64/34 <2-600 (35.8)	68/16 <2-530 (43.3)	20/2 <140-430 (285
Georgia	—	1/0	9/7 <1-51 (13.6)	—
Hawaii	1/0	1/0	—	—
Idaho	1/0	1/0	1/0	—
Illinois	—	1/1 (20)	—	1/0
Indiana	—	—	4/2 <9-61 (35)	—
Iowa	—	2/0	—	—
Kansas	1/0	1/0	2/0	—
Kentucky		NO ACTIVITY		
Louisiana	—	3/0	18/0	4/1 (5)
Maine	1/0	1/0	—	—
Maryland		NO ACTIVITY		
Massachusetts		NO ACTIVITY		
Michigan	1/1 (<2)		13/8 <4-60 (17.6)	15/4 <10-58 (29.5)
Minnesota	—	1/0	1/0	—
Mississippi	—	1/0	50/13 <3-56 (16.4)	—
Missouri		NO ACTIVITY		
Montana	4/0	6/0	4/0	—
Nebraska	1/0	3/0	1/0	—
Nevada	3/0	3/0	3/0	—
New Hampshire		NO ACTIVITY		
New Jersey	2/2 <5,<10	20/18 <3-4000 (230.2)*	15/7 <1-800 (172.2)	—
New Mexico	3/0	4/0	1/0	—

Table 2.1-3 (cont.)

STATE	1972	1973	1974	1975
New York	——	17/5	17/14	——
		<2-1300 (519)*	<2-450 (95.8)	
North Carolina	——	1/0	——	——
North Dakota	2/0	2/0	——	——
Ohio	——	1/0	——	——
Oklahoma	1/0	3/0	1/0	——
Oregon	1/0	1/0	2/0	——
Pennsylvania	9/7	5/3	23/16	3/3
	<5-E100 (50.7)	<5-20 (11.7)	<6-700 (105.6)	<42-240 (144)
Rhode Island	NO ACTIVITY			
South Carolina	2/0	2/0	5/1 (6)	——
South Dakota	1/0	1/0	——	——
Tennessee	——	1/0	1/0	——
Texas	41/3	58/8	58/17	——
	<4-120 (61.7)	<3-45 (17.6)	<1-153 (45.5)	——
Utah	——	3/0	1/0	——
Vermont	NO ACTIVITY			
Virginia	NO ACTIVITY			
Washington	1/0	3/0	2/0	——
West Virginia	NO ACTIVITY			
Wisconsin	——	9/1 (10)	21/0	2/0
Wyoming	——	3/0	1/0	——
Puerto Rico	——	6/0	16/5	——
			<10-640 (242)	

E - estimated

*mean figure does not include anomalous values

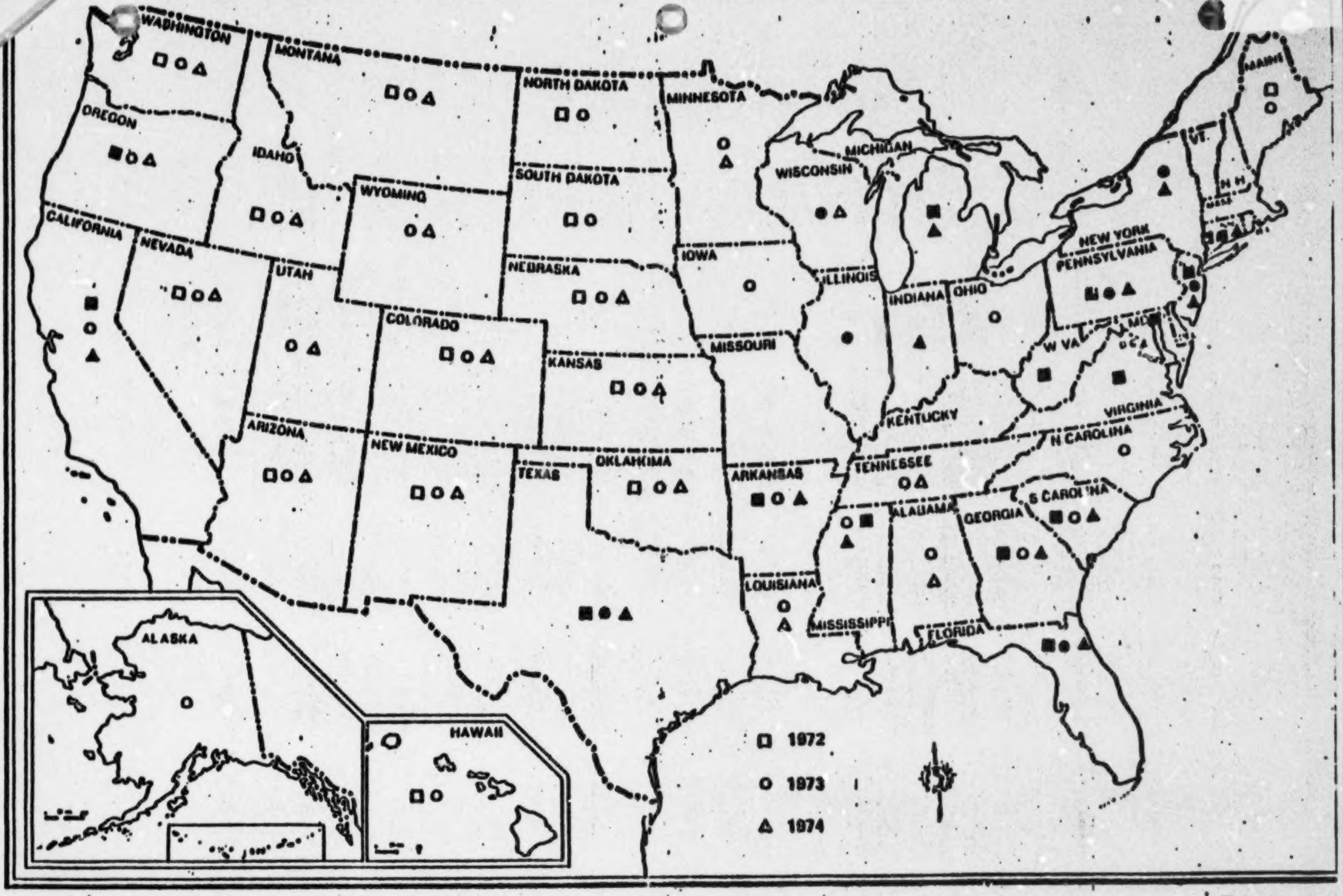
SOURCE: PCB Data Base, October 1972 to (partial) 1975, U.S. Geological Survey, Quality of Water Branch, Reston, VA.

Table 2.1-4
States Reporting PCB's in Bottom Deposits

1971-72	1973	1974	1975 (partial)
29/15 (51.72%)	39/8 (20.51%)	32/14 (43.75%)	6/4 (66.67%)

# states measuring PCB's in bottom sediments/# with at least one non-zero readin

SOURCE: PCB Data Base, October 1972 to (partial) 1975, U.S. Geological Survey, Quality of Water Branch, Reston, VA.



C-14

FIGURE 2.1-1 STATES MONITORING PCB's IN BOTTOM SEDIMENTS 1972-1974
● DARKENED SYMBOL REPRESENTS AT LEAST ONE DETECTION

2.1.3 Soils

Two national soils monitoring programs are conducted by EPA as part of its Pesticides Monitoring Program covering both cropland and urban soils. In 1973, the only year in which PCB's were analyzed in cropland soils, virtually no occurrences were detected[1].

The urban soils program is a small one, consisting of 5 different urban locations each year. In 1973, the only year for which PCB data in cropland soil are available, virtually no occurrences were detected. The sampling design recovers one analytical sample per square mile in the city proper and one per twenty square miles in the suburbs. The results of the program for the years 1971-1973 are summarized in Table 2.1-5 and Figure 2.1-2[1].

Among each year's set of 5 cites, 3 (60%) had at least one positive PCB site, cities and sites having been randomly selected. Over the three years an average of 2.68% of the sites in these cities (roughly corresponding to 2.68% of the total area) showed PCB concentrations in ranges from 0.9-11.94 ppm. The anomalous reading of 11.94 was found in a residential lawn in Gadsden, Alabama. Seventeen of the twenty two positive readings were below 1 ppm.

Table 2.1-5
National Urban Soil Monitoring Program
PCB Detections 1971 - 1973

	Total No. of Sites	No. of Positive Detections	Percent Positive Detections	Range of Positive Detections (ppm)	Arithmetic Mean (ppm)	PCB Type(s)
1971						
Baltimore, MD.	156	6	3.9	0.09 - 0.74	0.02	1260 1-lawn, 5 waste
Gadsden, AL.	55	1	1.8	11.94	0.21	Not identified, residential lawn
Hartford, CT.	48	ND	-			
Macon, GA.	43	ND	-			
Newport News, VA.	78	1	1.3	3.30	0.04	1254 lawn
1972						
Des Moines, IA	82	3	3.7	0.34 - 0.94	0.03	Not identified, 2-lawn 1-waste
Lake Charles, LA.	70	1	1.4	1.31	0.02	1254
Fitchburg, MA	35	ND	-			
Pittsburgh, PA.	189	1	0.5	1.01	0.01	Not identified, lawn
Reading, PA.	49	ND	-			
1973						
Evansville, IN.	82	ND	-			
Greenville, SC	86	3	3.5	0.13 - 1.59	0.02	1254 3-lawn
Pittsfield, MA.	45	ND	-			
Tacoma, WA.	95	6	6.3	0.18 - 0.63	0.03	1-1254, Others not identified, 2 lawn, 4 waste
Washington, D.C.	116	2	1.7	0.32 - 0.80	<0.01	1-1254, Others not identified, 1 lawn, 1 waste

SOURCE: Carey, Ann, 1975, EPA National Soil Monitoring Program, unpublished.

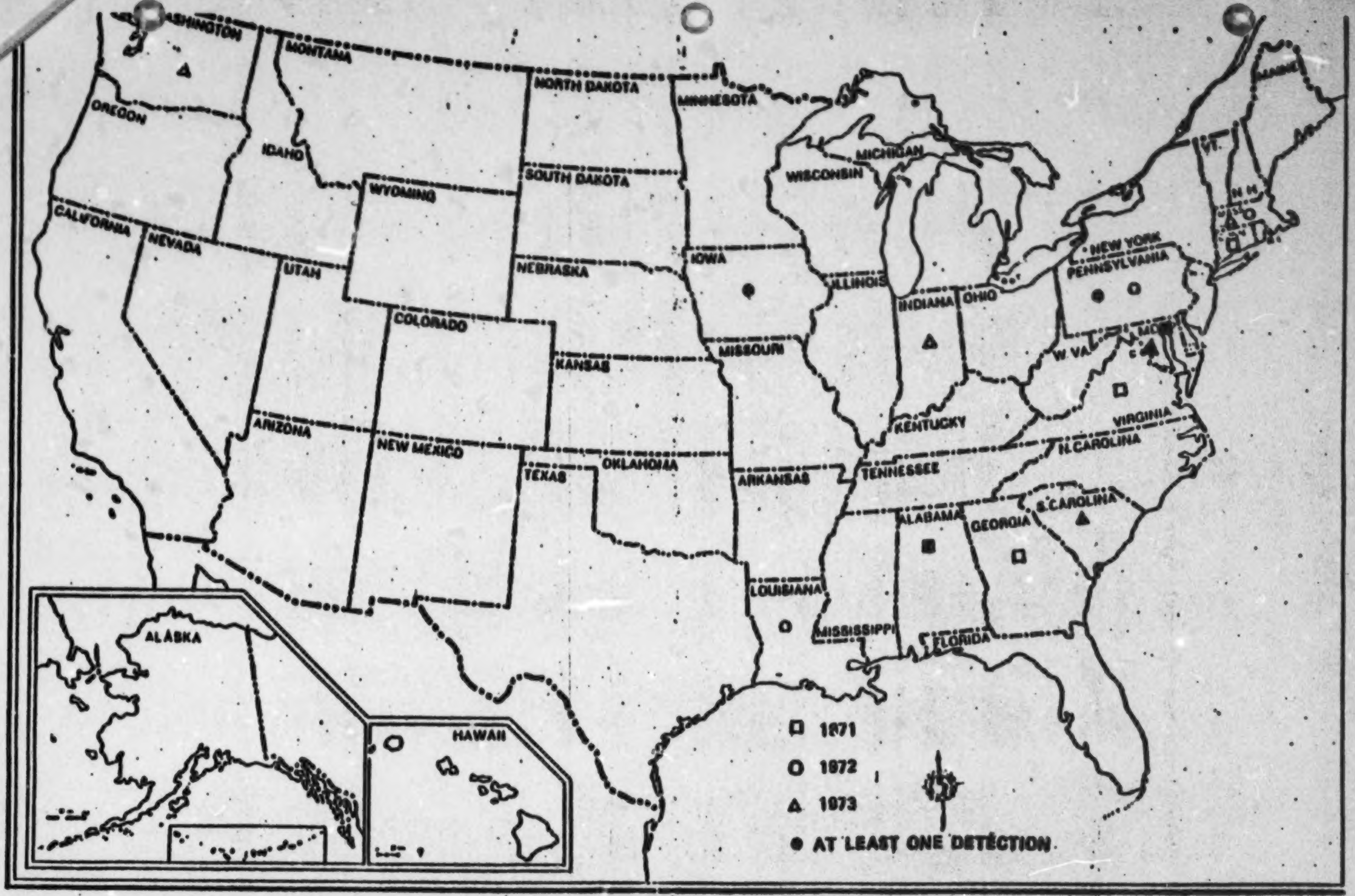


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FIGURE 2.1.2 NATIONAL URBAN SOIL MONITORING PROGRAM—PCB's

2.1.4 Air

Polychlorinated biphenyls have not been measured in air on a nationwide basis.

2.1.5 Fish

Fish have long been a popular sampling medium for PCB levels primarily due to the minimal difficulties of sampling and analysis relative to other media. Data on residue levels in fish, however, present problems of interpretation: the eating habits, lipid content, age, sex and size affect PCB levels; residue levels in fish, like those in sediment indicate past, not necessarily current, exposure; residue levels in fish are slow to reflect changes in levels of contamination of the water; distinguishing Aroclor 1242 from 1016 is difficult.

The U.S. Fish and Wildlife Service of the Department of the Interior provides the best national overview with the National Fish and Wildlife Monitoring Program. One of the elements of this program is the annual sampling of freshwater fish at 100 stations (as of 1970). Beginning with the 1969 collections, whole fish samples were analyzed for PCB's.

The most recent data from this program are the unpublished results of the 1973 collection. While many species of fish are sampled throughout the country, a reasonable summary of the data is provided in Table 2.1-6, which concentrates on the four most commonly sampled species of fish, largemouth bass, channel catfish, carp and yellow perch.

The great number of stations reporting "no detection" for those four species in 1973 is significant. Generally lower levels were reported for other species in 1973 also. Furthermore, when sampling data is totalled, ignoring both species and geographic location, as in Table 2.1-7, marked declines in both the proportion of composites with some PCB residues and the proportion with residues above the maximum recommended by Fish and Wildlife of 0.5 ppm are exhibited. While there is a downward trend in the number of stations reporting significant residue levels, those which have reported high concentrations in the past continue to do so. Walker[9] notes that PCB residues have been found in forty to sixty percent of the samples in residue concentrations exceeding 0.5 ppm and that the geographic dispersion shows that the higher concentrations appear to be associated with certain river systems having industrial activity: thirty-one of thirty-five stations in the Mississippi river system reported residues in excess of 0.5 ppm in the 1970-73 sampling program; all stations in the Great Lakes drainage area reported concentrations exceeding 0.5 ppm. Several factors related to interpretation of this data must be recognized: the PCB residues prior to 1973 are reported as "estimated", while those for 1973 are the result of the analysis for three Aroclors (1242, 1254 and 1260) totalled; a trend cannot be inferred from the data of one year, regardless of how dramatic it is, although such data may signal a turning point; analytic protocols for sample analysis, especially determination of specific Aroclors, are suspect and the results of cross check analyses show that some laboratories used for the analysis typically expressed PCB levels of only 5 to 20 percent of those discovered by more sophisticated analytic protocols of the Fish Pesticide Research Laboratory[9].

Table 2.1-6
PCB Residues in Selected Species of Fish
(averages of composite samples, expressed in ppm, wet weight, whole fish)

State	Waterway/ Station Location	Species	1969^a	1970^a	1971^a	1972^a	1973
AL	Alabama R.						
	Chrysler	Largemouth bass	<0.10	3.70	1.35	6.5	N.D.
		Channel catfish				1.5	
	Tombigbee R.						
	McIntosh	Carp		0.86	0.17		N.D.
		Largemouth bass		1.30	0.93	**	N.D.
		Channel catfish				**	
AZ	Colorado R.						
	Imperial Reservoir	Largemouth bass	0.40	0.11		N.D.	N.D.
		Carp	0.25	0.13	0.03	0.01	N.D.
		Channel catfish	0.64	0.17	0.17		
	Colorado R.						
	L. Havasu	Carp		0.17	0.05	N.D.	N.D.
		Largemouth bass		0.14		N.D.	N.D.
		Channel catfish		0.21	0.14		
	Colorado R.						
	L. Powell	Carp		0.09	0.06	<0.10	N.D.
		Largemouth bass		0.09	0.10	0.16	N.D.
	Gila R.						
	San Carlos Res.	Carp		0.04	0.08	**	N.D.
		Largemouth bass		0.08	0.03	**	N.D.
		Channel catfish			0.21	**	
AR	Arkansas R.						
	Pine Bluff	Carp	1.69	1.25	0.52	**	N.D.
		Channel catfish				N.D.	
CA	Sacramento R.						
	Sacramento	Carp	0.10	2.62	1.04	4.35	2.53
		Largemouth bass	<0.10	0.73	0.28	3.7	
	San Joaquin R.						
	Los Banos	Carp	0.10	3.74	0.12	**	N.D.
		Channel catfish	0.10	1.72	0.21		N.D.
				0.14	0.06		
	Klamath R.						
	Hornbrook	Yellow perch		0.24	0.12		<0.10
		Largemouth bass		0.09			
CO	Rio Grande						
	Alamosa	Carp		0.26	0.06	N.D.	
	Arkansas R.						
	John Martin Res.	Carp		0.09	0.05	0.05	N.D.
		Channel catfish		0.15	0.11		N.D.

Table 2.1-6 (cont.)

State	Waterway/ Station Location	Species	1969[a]	1970[a]	1971[a]	1972[a]	1973[b]
CT	Connecticut R. Windsor Locks	Yellow perch	3.40	2.45	17.1	6.6	5.6
FL	St. John's R. Welaka	Channel catfish	0.14	0.18	0.07	N.D.	N.D.
		Largemouth bass	0.31	0.37	0.28	N.D.	N.D.
	St. Lucie Canal Indiantown	Channel catfish	1.25	0.26	0.09	N.D.	N.D.
		Largemouth bass	0.56	0.62	0.08	N.D.	N.D.
	Suwanee R. Old Town	Largemouth bass		0.31	0.5	0.35	0.3
	Apalachicola R. Jim Woodruff Dam	Channel catfish	0.69	0.67	0.62		N.D.
		Largemouth bass	<0.10	0.53	0.12	2.5	N.D.
		Carp	<0.10			N.D.	
GA	Savannah R. Savannah	Largemouth bass	1.18	0.70	0.14	0.64	0.5
		Channel catfish				2.30	
		Carp	0.58	2.92	0.12		3.2
	Altamaha R. Doctortown	Largemouth bass		0.79	0.27	1.20	N.D.
ID	Bear R. Preston	Carp		0.89	0.82	1.9	0.8
		Yellow perch		1.29	0.42	1.0	2.35
	Salmon R. Riggins	Carp		0.14	0.06		
	Snake R. Lewiston	Carp		0.29	0.65	0.11	N.D.
IL	Ohio R. Metropolis	Carp		2.01	3.16	7.1	1.4
		Channel catfish		3.78	6.07	5.7	5.45
	Illinois R. Beardstown	Carp	11.3	0.82	0.64	2.1	1.45
IN	Wabash R. New Harmony	Carp		3.10	1.13	1.6	2.1
		Channel catfish		1.07	3.95	3.3	1.27
IA	Des Moines R. Keosauqua	Carp	0.54	0.22	0.16	1.33	1.00
		Channel catfish		0.40	0.86	1.60	0.90
		Largemouth bass				N.D.	
	Mississippi R. Guttenberg	Carp		0.30	1.21	1.0	1.2
		Largemouth bass	1.41	1.79	1.11	1.25	0.96

Table 2.1-6 (cont.)

State	Waterway/ Station Location	Species	1969^{a}	1970^{a}	1971^{a}	1972^{a}	1973^{b}
KS	Kansas R.						
	Bonner Springs	Carp		0.55	1.28	1.30	1.2
		Channel catfish			0.77		
LA	Mississippi R.						
	Luling	Carp	0.46	0.86	0.28	4.5	
		Channel catfish	0.66	0.58	1.95	6.6	
	Red R.						
	Alexandria	Channel catfish				N.D.	
		Carp					0.80
ME	Penobscot R.						
	Old Town	Yellow perch	0.31	0.33	0.30	0.36	0.3
	Kennebec R.						
	Hinckley	Yellow perch		0.23	0.15	0.20	N.D.
MD	Susquehanna R.						
	Conowingo	Carp	0.69	2.6	0.69	1.9	0.60
		Channel catfish	1.21	1.77	0.94	4.5	1.2
		Yellow perch	1.31	2.39	0.95	1.9	
	Potomac R.						
	Little Falls	Carp	1.04	0.63	0.81	1.1	
		Channel catfish					0.5
		Largemouth bass	1.04				N.D.
MA	Merrimac R.						
	Lowell	Yellow perch		6.12	21.2	9.5	12.0
MI	L. Huron						
	Bay Port	Carp		2.76	5.50		4.30
		Channel catfish		3.80	1.53		6.6
		Yellow perch		3.33	2.83	0.49	4.25
MN	Red R. (north)						
	Noyes	Channel catfish		0.68	2.29	0.17	N.D.
MS	Yazoo R.						
	Redwood	Carp		1.95	0.68	N.D.	N.D.
		Channel catfish			1.63		N.D.
MO	Mississippi R.						
	Cape Girardeau	Carp		5.26	1.89	2.42	1.9
		Largemouth bass		3.51	3.20		1.0
	Missouri R.						
	Hermann	Carp		1.29	7.6	2.8	4.6
		Channel catfish		0.21		2.00	

Table 2.1-6 (cont.)

State	Waterway/ Station Location	Species	1969[a]	1970[a]	1971[a]	1972[a]	1973[b]
MT	Big Horn R.						
	Hardin	Carp		0.42	0.05	N.D.	N.D.
		Channel catfish		0.55			N.D.
	Yellowstone R.						
	Sidney	Channel catfish		0.14			0.20
		Carp				0.10	N.D.
NE	Missouri R.						
	Nebraska City	Carp		1.38	0.53	0.67	0.30
		Channel catfish				0.50	
	North Platte R.						
	L. McConaughy	Carp		0.24	0.06	N.D.	N.D.
		Channel catfish		0.21	0.09	N.D.	N.D.
	South Platte R.						
	Brule	Carp		0.08	0.22	1.15	0.50
	Platte R.						
	Louisville	Carp		0.71	0.23	1.50	0.33
		Channel catfish		0.62	0.43	4.30	1.70
NV	Colorado R.						
	L. Mead	Carp		0.13	1.68	N.D.	0.20
		Largemouth bass		0.09	0.09	0.26	N.D.
		Channel catfish			0.13	0.76	0.20
	Truckee R.						
	Fernley	Carp	0.54	0.51	1,21	1.8	0.83
		Largemouth bass	<0.10	0.73	0.42	2.1	1.00
NJ	Raritan R.						
	Highland Park	Largemouth bass				4.9	
		Carp					2.1
NM	Rio Grande						
	Elephant Butte Res	Channel catfish		0.35	0.52	0.92	N.D.
		Largemouth bass		0.65	0.30	1.54	N.D.
NY	Hudson R.						
	Poughkeepsie	Largemouth bass	4.82	9.37	34.5	13.0	3.2
	St. Lawrence R.						
	Massena	Yellow perch		1.45	2.12	3.15	1.1
	L. Ontario						
	Port Ontario	Yellow perch		2.48	7.34	6.2	5.2
NC	Roanoke R.						
	Roanoke Rapids	Largemouth bass	0.10	0.89	0.37	1.40	N.D.
		Carp				0.65	N.D.
		Channel catfish					N.D.
	Cape Fear R.						
	Elizabethtown	Largemouth bass		3.33	0.81	3.90	N.D.

Table 2.1-6 (cont.)

State	Waterway/ Station Location	Species	1969[a]	1970[a]	1971[a]	1972[a]	1973[b]
ND	Missouri R. Garrison Dam	Carp	0.10	0.08		N.D.	N.D.
OH	Ohio R. Marietta	Carp		4.82	5.20	2.5	11.0
		Channel catfish		11.85	14.5	9.75	24.0
		Largemouth bass	8.07		22.65		
	Ohio R. Cincinnati	Carp		24.5	25.8	19.0	25.0
		Channel catfish			27.3	30.0	21.5
OK	Verdigris R. Oologah	Carp		0.61	0.8	**	N.D.
		Largemouth bass		0.40	0.09	**	N.D.
	Canadian R. Eufaula	Carp		0.81	0.31		N.D.
		Largemouth bass		0.14			N.D.
		Channel catfish			0.36		
	White R. DeValls Bluff	Carp		1.77	0.73	1.5	N.D.
		Channel catfish		1.82	2.32		N.D.
	Red R. Lake Texoma	Carp		0.25	0.26	N.D.	N.D.
		Largemouth bass		0.21	0.13	N.D.	N.D.
	Arkansas R. Keystone Res.	Carp	0.24	0.45	0.29	0.5	N.D.
		Largemouth bass	0.66	1.25		**	N.D.
		Channel catfish			1.28		
OR	Willamette R. Oregon City	Carp		1.25			
		Channel catfish				0.44	
	Columbia R. Bonneville	Carp			0.19	<0.1	N.D.
	Rogue R. Gold Ray Dam	Carp		2.60		1.4	5.4
		Largemouth bass		0.87			
PA	L. Erie Erie	Yellow perch		2.40	1.11	1.65	0.75
	Allegheny R. Natrona	Carp		4.59	5.52	4.0	1.75
SC	Pee Dee R. Dongola	Largemouth bass		0.62		3.70	0.5
	Cooper R. Summerton	Carp		1.04	0.35	N.D.	1.5
		Largemouth bass	<0.10	0.45	0.18	3.20	3.3

Table 2.1-6 (cont.)

State	Waterway/ Station Location	Species	1969[a]	1970[a]	1971[a]	1972[a]	1973[b]
SD	James R.						
	Olivet	Carp		0.20	0.65	N.D.	N.D.
		Channel catfish			0.20	1.20	<0.10
TN	Cumberland R.						
	Clarksville	Carp	0.89	1.67	0.69	16.0	4.3
		Largemouth bass	3.15	2.92	1.19	5.0	5.8
	Tennessee R.						
	Savannah	Carp		1.46	0.33	4.65	1.5
		Channel catfish		4.32	3.18		9.8
		Largemouth bass		1.20	0.63	11.0	3.1
	Mississippi R.						
	Memphis	Carp		3.26	1.22	10.0	1.5
		Channel catfish				5.4	
TX	Pecos R.						
	Red Bluff Lake	Channel catfish		1.07	0.19	0.60	N.D.
		Largemouth bass		0.06			
	Brazos R.						
	Richmond	Channel catfish		1.17		0.5	
	Colorado R.						
	Wharton	Channel catfish		0.32	0.38	1.1	N.D.
		Carp					N.D.
	Nueces R.						
	Mathis	Channel catfish		0.03		N.D.	
		Largemouth bass		0.05			N.D.
	Rio Grande						
	Brownsville	Channel catfish		0.40	3.62	7.70	3.46
UT	Utah Lake						
	Provo	Carp	0.29	0.18	0.10	**	0.26
	Green R.						
	Vernal	Carp	0.83	0.09	0.6	**	N.D.
		Channel catfish		0.10	0.22	**	N.D.
VT	L. Champlain						
	Burlington	Yellow perch		0.78	0.74	1.20	1.21
VA	James R.						
	Richmond	Channel catfish		0.79	0.44		1.5
		Largemouth		0.34	0.33		1.5
WA	Snake R.						
	Ice Harbor	Channel catfish		1.00	0.62	1.3	0.5
		Carp			0.19		
	Yakima R.						
	Granger	Carp		1.24	0.22	1.70	N.D.
		Largemouth bass		0.63			N.D.
	Columbua R.						
	Pasco	Carp		0.17	0.19	1.4	N.D.

Table 2.1-6 (cont.)

State	Waterway/ Station Location	Species	1969	1970	1971	1972	1973
WV	Kanawha R. Winfield	Carp	0.31	1.25	0.61	12.0	3.8
WI	L. Michigan Sheboygan	Yellow perch		7.42	11.45	9.3	7.85
	Wisconsin R. Woodman	Carp		1.15	1.44		3.67
		Channel catfish		0.17	1.11		

** - PCB-like compound present
N.D. - Not Detectable

a. estimated PCB levels
b. total of Aroclors 1242, 1254 and 1260

SOURCES:

1969 Data - Henderson, C., A. Inglis and W.L. Johnson, Pesticides Monitoring Journal, 5, 1-11 (1971).

1970-1973 Data - National Fish and Wildlife Monitoring Program, Results of Freshwater Fish Monitoring 1970-1973, U.S. Fish and Wildlife Service, U.S. Department of Interior, Washington, DC (unpublished).

Table 2.1-7
Summary of PCB Residues Detected in Fish from 1969-1973
National Pesticide Monitoring Program

		Number of Composites				
		With Residues		With Residues Above 5 ppm		
Year	Total	No.	%	No.	%	Range (ppm)
1969	147	147	100	89	61	<0.10-14.8
1970	393	390	99	239	61	0.03-24.8
1971	587	581	99	284	48	0.02-41.0
1972	401	311	78	215	54	<0.05-30.0
1973	400	202	51	160	40	<0.01-25.0

SOURCES:

1969 - Henderson, C., A. Inglis and W.L. Johnson, Pesticides Monitoring Journal, 5, 1-11 (1971).

1970-1973 Data - National Fish and Wildlife Monitoring Program, Results of Freshwater Fish Monitoring 1970-1973, U.S. Fish and Wildlife Service, U.S. Department of Interior, Washington, DC (unpublished).

2.1.6 Birds

Two of the three elements of the National Fish and Wildlife Monitoring Program mentioned in the previous section are: mallards and black ducks; starlings.

PCB's have been found in all of the starling samples taken in 1970, 1972 and 1974. The level of residues appears to be declining over this period with national arithmetic means of 0.65 ppm, 0.43 ppm and 0.12 ppm for the three years respectively[9].

Results of sampling of mallards in 1970 and 1972[9] are shown by flyway on Figure 2.1-3. Residues are highest in the Atlantic flyways, with average concentrations more than double that of any other flyway and more than 10 times greater in some cases. Samples of black ducks were also taken in the Atlantic flyways in 1969 and 1972 and the mean PCB concentration was constant at 1.36 ppm.

In the 1969 mallard study[4] not only did mallards from the Atlantic flyway show the greatest average PCB concentration, but with the exception of Maryland all of the states in the Atlantic flyway had averages exceeding 0.5 ppm with the highest residues from New Hampshire southward through Delaware. In the Mississippi flyway, residue levels were highest in states bordering the Great Lakes, generally diminishing as sampling moved southward.

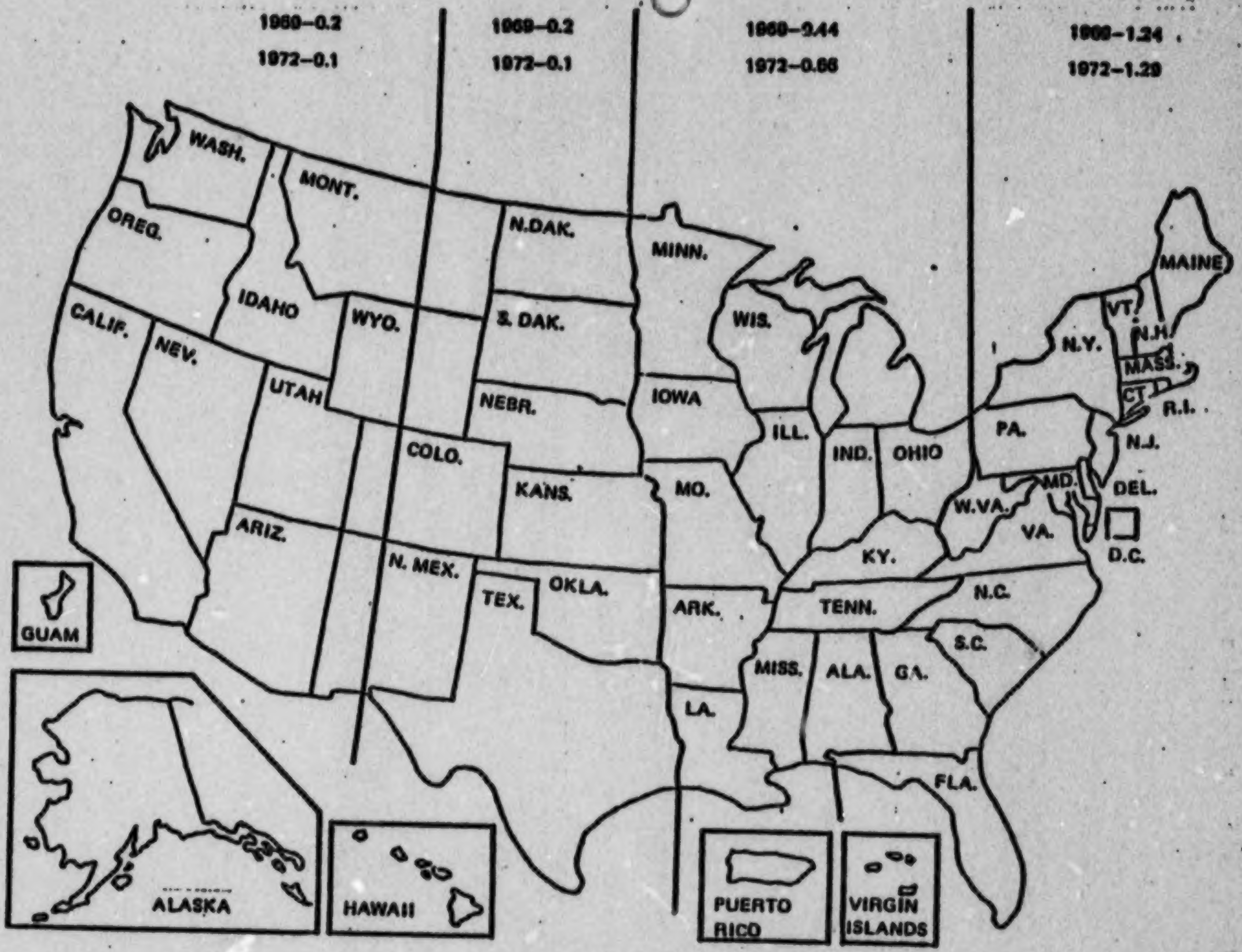

FIGURE 2.1-3 PCB's IN MALLARDS (AVERAGES, WINGS, PPM, WET WEIGHT BASIS)

REFERENCES, Section 2.1

1. Carey, Ann, 1975, EPA National Soil Monitoring Program, unpublished.

2. Crump-Wiesner, J.S., H.R. Feltz and M.L. Yates, J. Research USGS, 1, 603-607 (1972).

3. Haile, C.L., G.D. Veith, G.F. Lee and W.C. Boyle, Chlorinated Hydrocarbons in the Lake Ontario Ecosystem (IFYGL 1975), unpublished EPA report, EPA-660/3-75-022.

4. Heath, R.G. and S.A. Hill, Pesticides Monitoring Journal, 7, 153-163 (March 1974).

5. Henderson, C., A. Inglis and W.L. Johnson, Pesticides Monitoring Journal, 5, 1-11 (1971).

6. Martin, W.E. and P.R. Nickerson, Pesticides Monitoring Journal, 6, 33-40 (June 1972).

7. National Fish and Wildlife Monitoring Program, Results of Freshwater Fish Monitoring 1970-1973, U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington, DC (unpublished)..

8. PCB Data Base, October 1972 to (partial) 1975, U.S. Geological Survey, Quality of Water Branch, Reston, VA.

9. Walker, Charles R., The Occurence of PCB's in the National Fish and Wildlife Monitoring Program, presented at the National Conference on Polychlorinated Biphenyls, Chicago, Illinois, November 19-21, 1975.

2.2 Data from Localized Monitoring Efforts - Lakes

Figure 2.2-1 is provided in order to display the relationship of the different lakes in the Great Lakes region as discussed in 2.2.1 - 2.2.5.

2.2.1 Lake Ontario

Lake Ontario is one of the lesser studied Great Lakes for PCB's. A thorough study of chlorinated hydrocarbons in the Lake Ontario ecosystem was performed in 1972 by Haile, Veith, Lee and Boyle.[6] Samples of various media were taken from 19 different locations in Lake Ontario during the summer of 1972, as indicated in Figure 2.2-2, and significant concentrations of PCBs were reported in all media. Findings for water, sediment and net plankton are summarized in Table 2.2-1. Other than the south shore areas off Oswego (77 ppt) and the north of the Niagara River (97 ppt), the PCB content of the Lake Ontario water appears relatively uniform in the range of 35 to 56 ppt, with an average of 45 ppt. PCB concentrations in sediments around the south shore areas off the mouths of the Welland Canal and Niagara River and off Oswego averaged more than twice the concentrations found at the four other sites (means of 184 ppb vs. 72 ppb). This led the authors to conclude that the Niagara and Oswego Rivers may be important sources of PCB associated with settleable particulates. Plankton samples were not taken at the south shore sites; lake wide levels averaged 7.2 ppm.

Fish samples selected in the same study found mean PCB concentrations on a whole fish basis in smelt (2.65 ppm) similar to those reported in Lake Michigan smelt. Mean PCB levels in alewives (2.35 ppm) were lower than values reported for Lake Michigan alewives (4.6 ppm). Bottom feeding slimy sculpin, which are less migratory than the latter two species, exhibited greater station to station variability in PCB levels, with a lake wide average of 4.63 ppm. Average concentrations in Cladophora were 515 ppb and for benthic fauna were 471 ppb. Samples were not taken consistently across media at stations and the results suffer accordingly, but the authors do draw one of the few estimates of accumulation factors for chlorinated hydrocarbons. They estimate relative concentrations as 1; 2500; 10,000; 150,000; and 300,000 for water, sediment, benthos, net plankton and fish, respectively.

Kaiser[10] shows PCB levels in two 1973 fish samples from northern Lake Ontario as 5.99 ppm (total PCB, fat, northern longnose gar) and 2.38 ppm (total postanal fins, northern pike), magnitudes considered comparable to those of other fishes from Lake Ontario.

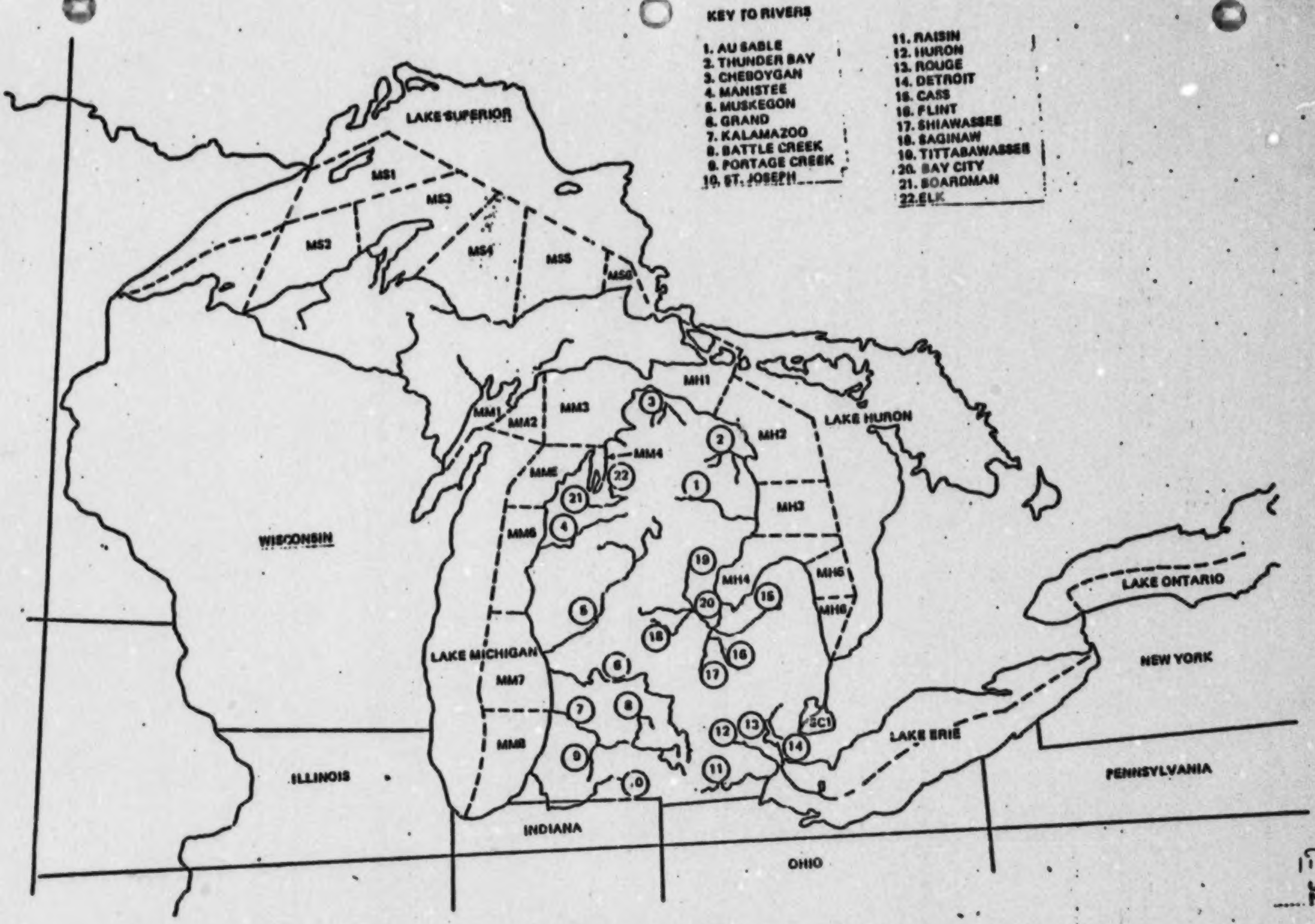

FIGURE 2.2-1 THE GREAT LAKES (AND RIVERS IN MICHIGAN)

Table 2.2-1

PCB Concentrations, Lake Ontario Ecosystem
Summer 1972

Sample Location (map reference)	Water (ppt)	Sediment (ppb dry)	Net Plankton (ppm dry)
1	49		3.4
8	35		
10			10.6
12		245	
13	97	155	
30	44	80	
36	45	43	7.6
45			3.6
46		79	
60	40	84	
75	56		11.8
90	77		
91		158	
96			6.0

SOURCE: Haile, C.L., G.D. Veith, G.F. Lee and W.C. Boyle, Chlorinated Hydrocarbons in the Lake Ontario Ecosystem (IFYGL), EPA Report. EPA 660/3-75-022 (1975).

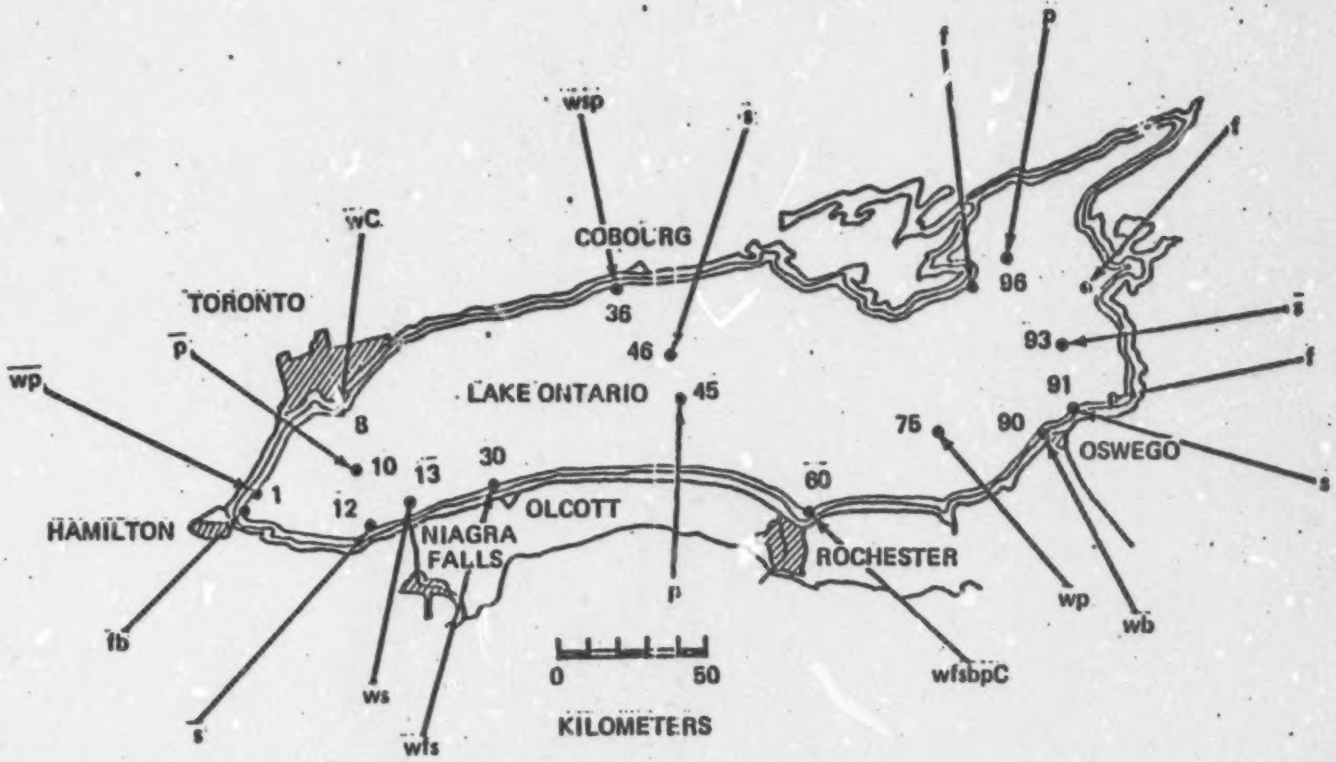

FIGURE 2.2-2 LAKE ONTARIO SAMPLING SITES FOR CHLORINATED HYDROCARBON ANALYSIS. NOTATIONS ARE: f-FISH, w-WATER, s-SEDIMENT, p-NET PLANKTON, C-CLADOPHORA, b-BENTHOS. STATION NUMBERS ARE IFYGL STATION IDENTIFIERS.

2.2.2 Lake Erie

Several studies of PCB concentrations in fish samples from Lake Erie are summarized in Table 2.2-2. Levels are generally lower than those reported from Lake Michigan and Lake Superior, but are inconclusive other than demonstrating the presence of PCB's in the lake water. Water measurements in tributaries to Lake Erie[3] including the Rouge river, which is tributary to the Detroit River are in Table 2.2-3, the Raisin and Rouge Rivers show relatively high levels of concentration when contrasted to the concentration levels in fish in the preceding table.

Table 2.2-2

Mean PCB Levels in Fish, Lake Erie
(ppm, whole fish)

Species	1970[a]	1972[b]	1973[c]
Carp	2.0		9.3
Catfish	4.4		
Drum	1.1		0.9
Yellow Perch	0.8	0.25	Trace
Salmon	2.1		
Walleye			0.5
White Bass	2.1	1.02	2.5
Smallmouth Bass		0.71	

SOURCES: [a] Carr, R.L., C.E. Finsterwalder and M.J. Schibi, Pesticides Monitoring Journal, 6, 23-26 (1972).

[b] Kelso, J.R.M. and R. Frank, Transactions American Fisheries Society, 103, 577-581 (1974).

[c] 1973 Great Lakes Environmental Contaminant Survey - Data Summary and Analysis, Bureau of Consumer Protection, Michigan (1973).

Table 2.2-3

PCB Concentrations in Michigan Tributaries to Lake Erie in 1971-72 and June 1973 in ppb as Aroclor 1254

River	Mean PCB Concentration	Range (1971-72)	1973
Raisin	0.210	<0.068-0.500	<0.010
Huron	0.012	<0.010-0.039	<0.010
Rouge	0.470	0.058-1.600	0.460*
Detroit	0.020	<0.010-0.053	<0.010

* Aroclor 1242

SOURCE: Hesse, J.L., Status Report on Polychlorobiphenyls in Michigan Waters, Michigan Water Resources Commission, June 1973.

2.2.3 Lake Superior

The results of several available fish studies are presented in Table 2.2-4 for different species of fish. Lake trout are in the highest trophic level within the lake and biological accumulation should result in this species possessing some of the highest concentrations of PCB's.

The study by Veith and Glass[19], summarized in the table, drew samples from three areas in the western portion of the lake. There was variability in the levels of PCB's in fish caught in different areas, but no conclusions are drawn in the study. Samples from the Apostle Island region (Wisconsin) had higher mean concentrations for all species than those from the other two regions (Minnesota). Parejko and Johnston[14] note that the biphenyls containing lower percent concentrations of chlorine predominated in the sample. The latter study involved tests for correlation between factors of age, sex, geographical location and concentration level; surprisingly, no significant correlations were discovered.

The geographic distribution of lake trout samples from the 1972-1974 Great Lakes Environmental Contaminant Survey[5] is detailed in Table 2.2-5.. As regards recent screening of fish against the FDA guideline, Kleinert[12] reports that of 64 fish samples collected in the nearshore waters during 1974, none exceeded the 5 ppm guideline and only two exceeded a level of 1 ppm.

Table 2.2-4

Mean PCB Levels in Fish, Lake Superior
(ppm, whole fish)

Species	1971[c]	1972[a]	1973	1974[e]
Burbot		0.7	1.4[a]	
Chubs		0.9	1.6[b]	
Herring		2.0	1.8[b]	
Lake Trout	7.08	0.8	1.8[a] 2.64[d]	1.55
Long-Nose Sucker			0.9[a]	
Smelt		0.3	0.4[a]	
Whitefish			0.3[b]	

SOURCES:

[a] Veith, G.D. and G.E. Glass, PCBs and DDT in Fish from Western Lake Superior, U.S. EPA National Water Quality Lab., Duluth, Minnesota (1974).

[b] 1973 Great Lakes Environmental Contaminant Survey - Data Summary and Analysis, Bureau of Consumer Protection, Michigan (1973).

[c] Parejko, R. and R. Johnston, Uptake of Toxic Water Pollutants, (PCB) by Lake Trout. Project Completion Report, U.S. Dept. of the Interior, Office of Water Resources Research, Contract 14-01-0001-3522 (1973).

[d] Willford, W.A., Contaminants in Upper Great Lakes Fishes, Presented at Great Lakes Fishery Commission, Upper Great Lakes Committee Meetings, Milwaukee, Wisconsin, March 1975.

[e] Hesse, J.L., PCB Situation in Great Lake Fish, Report to Michigan Water Resources Commission, April 17, 1975.

Table 2.2-5
Mean PCB Levels in Lake Trout from Lake Superior (ppm)

Lean Variety		<17"	17-20"	20-25"	>25"
MS 1**	1972	—	—	—	—
	1973	2.4 (4)*	2.9 (1)	5.6 (3)	4.8 (4)
	1974	—	—	—	—
MS 2	1972	—	—	—	—
	1973	0.5 (3)	1.8 (3)	3.4 (3)	5.2 (2)
	1974	—	—	1.2 (3)	5.2 (1)
MS 3	1972	—	—	2.2 (6)	2.7 (3)
	1973	1.6 (12)	1.9 (7)	4.5 (8)	4.8 (11)
	1974	0.6 (3)	0.1 (2)	0.4 (9)	2.0 (12)
MS 4	1972	—	—	4.6 (3)	5.7 (8)
	1973	—	1.0 (6)	2.0 (6)	4.7 (9)
	1974	0.5 (2)	0.5 (2)	0.7 (4)	1.4 (3)
MS 5	1972	—	—	3.3 (6)	2.1 (3)
	1973	—	1.2 (3)	1.2 (3)	1.9 (3)
	1974	—	1.8 (2)	1.4 (4)	1.5 (6)
MS 6	1972	—	—	1.4 (3)	—
	1973	0.9 (3)	1.0 (3)	1.5 (3)	1.3 (3)
	1974	2.2 (8)	0.4 (2)	2.8 (7)	2.6 (5)
Fat or Siscowet Variety					
MS 1	1974	0.5 (3)	3.6 (2)	1.9 (4)	3.8 (3)
MS 2	1974	2.5 (5)	6.2 (10)	11.3 (6)	3.3 (2)
MS 3	1974	4.4 (6)	12.6 (2)	7.4 (7)	6.8 (7)
MS 4	1974	12.7 (2)	5.4 (8)	—	6.1 (6)

*Concentrations in parts per million (ppm) in fillet; wet weight. Number of samples in parentheses ( ).

**Refers to geographic area in Figure 2.2-1.

SOURCE: Hesse, J.L., Contaminants in Great Lakes Fish, Staff Report, Michigan Water Resources Commission, Department of Natural Resources, (1975).

2.2.4 Lake Michigan

Lake Michigan is the most heavily studied of the Great Lakes. It has been established that the lake contains the highest concentrations of agricultural pesticides due, in part, to the large useage in the watershed relative to the flushing period for the lake and to the relatively low biomass density. There were seizures of Lake Michigan fish by the FDA during 1975. Samples from a shipment of Lake Michigan Chinook and Coho Salmon in May 1975 were found to exceed the 5 ppm guideline.[2] PCB levels in these fish ranged from 7.6-10.9 ppm. Hesse[7] reports a February 1975 seizure of large, surplus salmon exceeding the FDA guideline. In May 1975[2] a seizure of chubs resulted in analysis which showed that concentrations did not exceed the guideline. Such activities, however, have dramatized the hazards to both the lake and its fishing industry. In June, 1975, the Secretary of the State of Wisconsin Department of Natural Resources, based on evidence indicating that most large trout and salmon contain PCB's at levels exceeding the 5 ppm tolerance level, concluded that most fish over 24 inches in length appear to have excessive levels.

To provide a broad historical perspective, data from several studies of Lake Michigan fish are assembled in Table 2.2-6. The question of trend in the levels of fish contamination has been addressed by both Hesse[8] and Willford[21], referring specifically to the data of Table 2.2-7. Both conclude that the situation has generally remained static since 1972. Statistical analyses show no significant differences in the levels of the three species over the three year period 1972-1974. Data from the Great Lakes Environmental Contaminant Survey for this period for lake trout is detailed, by sampling area, in Table 2.2-8. The sample sizes are generally small and, while not allowing for rigorous statistical analysis, document the presence of a widespread PCB problem in lake trout of larger sizes and an intensification of the problem in southern Lake Michigan. The general conclusions that larger fish such as brown, lake and rainbow trout and chinook and coho salmon contain PCB residues at levels exceeding the FDA guideline, that concentrations increase with the percentage of fat and the size of the fish, and that levels found in the southern portion of the lake exceed those in the northern portion are supported by various studies[2].

The results of some earlier (1971-72) fish sampling undertaken by the Illinois EPA[3] found PCB levels in the edible portion of five species (yellow perch, chub, carp, coho salmon and alewife) generally below the 5 ppm tolerance limit. This is in apparent contradiction to the later studies discussed in the preceding paragraph - especially since the Illinois samples came from the southern portion of the lake. Data from this study is included in Table 2.2-6. There are, of course, many variables which impact on the interpretation of data from fish sampling lipid content, fish size and age, season of capture, location, etc. Thus, mean readings may not be truly representative although this statistic is commonly used to provide broad summarization of an abundance of data.

Measurements taken on Lake Michigan open waters and sediment tend to show results which are consistent with reported sediment data from other sections of the country (see section 2.1.3), and somewhat higher readings for concentrations in whole water. The Illinois EPA has found tributary sediments with higher concentrations than those from open waters (1971): averages of 23.06 ppb as Aroclor 1242 and 14.66 ppb Aroclor 1254 for the former contrasted with 95.77 ppb Aroclor 1242 and 32.27 ppb Aroclor 1254 for the latter[4]. Whole water concentrations in tributaries analyzed as Aroclor 1242 or 1254 ranged from 0.1 to 4.0 ppb during the period 1971-1974[2]. PCB concentrations measured by the Illinois State Board of Health in tributaries to Lake Michigan during 1973-1974 ranged from 0.01 to 0.09 ppb.

The Milwaukee River, a Wisconsin tributary to Lake Michigan was sampled in August 1969 for PCB's[20]; concentrations of Aroclors 1260 and 1242 were in the ranges trace to 0.26 ppb and trace to 2.07 ppb, respectively. The high Aroclor 1242 reading was validated by a repeat measurement (of 2.80 ppb in February 1970) and attributed to combined sewer outfalls and contaminated industrial cooling waters. Estimates of PCB concentrations in sewage treatment plant effluents in March, 1970, ranged from 0.04 ppb to 0.25 ppb, but the effluent from a chemical plant had a concentration of 2.50 ppb. This led the authors to conclude that PCB's were discharged to natural waters through municipal and industrial wastes and that PCB's in such large ecosystems as Lake Michigan have resulted in part, through water transport from metropolitan areas. This certainly is true; unfortunately no follow-up studies were performed to estimate the effect of both controls and increased attention to the PCB problem.

With the exception of a contamination problem in the Kalamazoo River, most PCB measurements in Lake Michigan tributaries within the state of Michigan are at levels in whole water of less than 0.1 ppb. Data showing levels identified in 1971-1972 are in Table 2.2-9. The Kalamazoo River was the subject of studies in both 1971 and 1972[8]. Concentration levels were higher downstream from Battle Creek, and some sources of contamination were identified.

Fifty four fish samples were collected from 15 stream sections on the Kalamazoo River in July 1971[4]. Measurable concentrations were found in nearly all fish samples tested with concentrations ranging from less than 0.01 to 109.9 ppm in edible portions of fish on a wet weight basis. Those fish from the north and south branches and the main stream of the river down to Battle Creek had low concentrations ranging from 0.01 to 0.33 ppm. Downstream from the Battle Creek wastewater treatment plant the levels in fish increased ranging from 0.82 to 18.75 ppm and remained high downstream with levels below Kalamazoo ranging from 1.35 to 109.9 ppm[4].

Settleable solids were only sampled at eight stations in 1971 with levels identified ranging from 0.01 to 0.422 ppm[4]. The levels in the mainstream were 10 to 20 times higher than those in the north and south branches and gradually increased downstream. The sample from the Battle Creek River had the highest concentration in the watershed indicating that the tributary contributes significantly to the total problems in the mainstream.

Since the Kalamazoo River is the most polluted input to Lake Michigan further sampling to identify possible sources of PCB loss to the environment was conducted in the vicinity of the City of Kalamazoo in 1972[15]. PCB concentrations from the discharge of several industries and a wastewater treatment plant were below the limit of detectability. However, Portage Creek, which receives effluents from several industries including two paper mills, had 0.47 ppb of Aroclor 1242. Settleable solids that were collected showed concentrations ranging from 0.23 to 2.63 ppm at eight of the ten stations. The two stations that did not have significant concentrations were upstream from the paper mill ponds. Sediment core samples taken from the Bryant paper mill ponds had concentrations up to 368.7 ppm as deep as six to eight inches.[5]

Further data collected in the spring of 1973 on settleable solids are presented in Table 2.2-10 demonstrating considerable sensitivity of measurements to sampling intervals. The levels detected in 1975 were also generally higher for the Kalamazoo River than were detected in 1971.

Table 2.2-6
Mean PCB Levels in Lake Michigan Fish
(ppm, whole fish)

Species	1971[b]	1972[d]	1973[f]	1974[e]
Alewife	2.84[a], 4.6	2.4		
Brown Trout	7.3			
Carp	2.04[a], 4.2	3.9		
Chub	2.85[a], 6.0	7.0, 2.83[a]	4.48	
Chinook Salmon	11.4	12.4	6.4	
Coho Salmon	1.68[a], 11.5	11.2, 10.93[e]	4.9, 12.17[e]	10.45
Lake Trout	13.53[c], 15.5	7.4, 12.86[e]	18.93[e]	22.91
Perch	5.8	0.4		
Rainbow Trout	9.3			
Red Sucker	3.0			
Smelt	2.7		1.6	
White Sucker	3.9			
Whitefish	3.0	0.7	0.9	
Menominee		1.1	1.0	
Suckers		1.0	0.45	
Steelhead		6.0		
Bloater Chubs		5.66[e]	5.24[e]	5.57
Yellow Perch	0.22[a]	0.3[a]	0.4	

SOURCES:

[a] U.S. EPA, Pesticide Monitoring Programs: Lake Michigan and Tributaries in Illinois, EPA 600/3-74-002.

[b] Veith, G.D., Chlorinated Hydrocarbons in Fish from Lake Michigan, University of Wisconsin, Madison, Wisconsin, unpublished report on EPA Project 16020 PBE, (1973).

[c] U.S. EPA, An Evaluation of DDT and Dieldrin in Lake Michigan, EPA-R3-72-003, (1972).

[d] Hesse, J.L., Status Report on Polychlorobiphenyls in Michigan Waters, Michigan Water Resources Commission, (June 1973).

[e] Bremer, K.E., State of Concerns of the Lake Michigan Toxic Substances Committee Related to Polychlorinated Biphenyls (draft) U.S.EPA, (1975).

[f] 1973 Great Lakes Environmental Contaminant Survey-Data Summary and Analysis, Bureau of Consumer Protection, Michigan, (1973).

Table 2.2-7
Concentrations of PCBs in fall collections of Lake Michigan Bloaters and Lake Trout off Saugatuck, Michigan, and Coho Salmon from between Ludington and Frankfort, Michigan

Species and Year	Number of Fish	Average Length (mm)	Total PCBs[b] (ppm)
Bloaters			
1972	120[c]	255	5.66 (0.95)
1973	160[c]	250	5.24 (0.37)
1974	110[c]	257	5.57 (0.31)
Coho salmon			
1972	10	693	10.93 (2.12)
1973	29	620	12.17 (0.77)
1974	30	665	10.45 (0.92)
Lake trout			
1972	9	648	12.86 (4.75)
1973	30	602	18.93 (2.08)
1974	30	616	22.91 (3.73)

[a] Analysis performed by Great Lakes Fishery Laboratory.

[b] Concentrations in whole fish, wet weight with 95% confidence interval in parentheses.

[c] Composite samples, 10 fish per sample.

SOURCE: Bremer, K.E., State of Concerns of the Lake Michigan Toxic Substances Committee Related to Polychlorinated Biphenyls (draft) U.S. EPA, (1975).

Table 2.2-8
Mean PCB Levels in Lake Trout from Lake Michigan (ppm)

		<17"	17-20"	20-25"	>25"
MM 1**	1972	—	—	—	—
	1973	2.2 (1)*	3.6 (2)	—	—
	1974	0.7 (1)	1.6 (2)	—	—
MM 2	1972	—	—	—	—
	1973	—	—	—	—
	1974	—	—	—	—
MM 3	1972	—	—	—	11.1 (3)
	1973	2.6 (3)	2.4 (3)	—	10.6 (3)
	1974	—	—	—	—
MM 4	1972	—	—	—	6.5 (3)
	1973	2.6 (2)	1.8 (1)	—	8.5 (3)
	1974	1.0 (3)	3.1 (3)	—	—
MM 5	1972	—	—	—	—
	1973	—	—	5.8 (1)	7.3 (2)
	1974	—	—	7.5 (6)	9.6 (6)
MM 6	1972	2.4 (1)	—	—	4.1 (1)
	1973	—	6.4 (1)	7.1 (2)	11.0 (3)
	1974	1.1 (9)	2.5 (16)	5.6 (11)	9.6 (18)
MM 7	1972	2.7 (2)	—	6.8 (1)	7.3 (3)
	1973	—	—	—	11.2 (3)
	1974	2.4 (9)	—	8.9 (8)	11.9 (2)
MM 8	1972	—	5.8 (2)	6.4 (1)	12.4 (3)
	1973	5.1 (6)	8.2 (3)	10.1 (4)	12.6 (4)
	1974	—	—	—	—

*Concentrations in parts per million (ppm) in fillet; wet weight. Number of samples in parentheses ( ).

**Refer to geographic area in Figure 2.2-1.

SOURCE: Hesse, J.L., Contaminants in Great Lakes Fish, Staff Report, Michigan Water Resources Commission, Dept. of Natural Resources (1975).

Table 2.2-9
Mean PCB Concentrations in Michigan Tributaries to the Lake Michigan Basin, 1971-72 and 1973
(ppb as Aroclor 1254)

River	1972-72		1973
	Average	Range	
St. Joseph	0.013	<0.010-0.039	<0.010
Kalamazoo	0.065	0.019-0.097	0.040
Grand	0.041	0.011-0.080	0.033
Muskegon	0.010	<0.010-0.C37	<0.010
Manistee	0.014	<0.010-0.039	0.037
Boardman	0.017	<0.010-0.044	0.018
Elk	0.012	<0.010-0.039	<0.010

SOURCE: Hesse, J.L., Status Report on Polychlorobiphenyls in Michigan Waters, Michigan Water Resources Commission, (June 1973).

Table 2.2-10
Concentrations of PCB's (polychlorinated biphenyls) in the settleable solids collected from the mouths of the Grand, Kalamazoo and St. Joseph Rivers; Spring 1973, for comparison of variable sampling periods. Concentrations in ppm on an oven-dry basis.

River	Sample Type[a]	Sample Dates 3/21	3/23	3/30	4/2	4/4	4/6	4/9	4/11	4/13	4/20	4/27	4/30	5/2	5/4	5/11	Means
Grand	3X	3.24			1.6*	0.95	1.0	<0.1	0.93	1.58			0.83	3.5	0.53		1.43
	Weekly		1.25	0.94			1.1			1.0	0.55	0.74			0.80	0.77	0.89
	Biweekly			0.85						0.73		0.50				0.60	0.67
	Monthly									1.41						0.50	0.96
																Mean =	0.99
Kalamazoo	3X	6.7	27.5		2.08	<0.1	2.52	1.33	3.20	<0.1			3.11	3.50	3.75		4.72
	Weekly		6.83	3.0			3.54			3.77	3.09	2.98			2.88	4.87	3.87
	Biweekly			3.77						2.54		2.64				2.50	2.86
	Monthly									2.11						3.14	2.63
																Mean =	3.52
St. Joseph	3X												0.63	0.82			0.73
	Weekly								2.13	0.68		0.95		0.83	1.21		1.16
	Biweekly									0.85		0.75			0.95		1.24
	Monthly											0.67			0.83		0.75
																Mean =	0.97

[a] 3X = Sample collected Mon, Wed. and Friday
*Concentration based upon a 1:1 ratio of Aroclor 1242 and 1251.
All other concentrations calculated as Aroclor 1242.

SOURCE: Monitoring for Polychlorinated Biphenyls in the Aquatic Environment, Michigan Water Resources Commission, (May 1973)

2.2.5 Lake Huron

Levels of PCB's in fish samples from Lake Huron are detailed in Table 2.2-11 from areas as indicated in Figure 2.2-1. The Saginaw Bay area, designated as MH-4 on the map, has been the object of detailed analysis, and significant concentrations have been detected. Water measurements in tributaries to Lake Huron, including the Saginaw Bay area, are in Table 2.2-12. Concentrations in fish taken from the Saginaw River in 1971 were as high as 165 ppm as shown in Table 2.2-13. The waste water treatment plant on the Saginaw River is the Bay City facility. During 1971, this plant had an average effluent concentration of 120 ppb with a high of 340 ppb.

After control measures were initiated by some industrial sources in October 1972, there was a sharp drop in PCB levels at the mouth of the river. Levels measured in 1972 ranged from <0.020 to 0.640 ppb and in 1973 from <0.100 to 0.200 ppb. This is one of the noteworthy examples of controls effecting a marked change in PCB contamination[8].

Table 2.2-11
Mean PCB Levels, Whole Fish in Lake Huron (ppm)

Species	1973
Brown Trout	3.6 (MH-2)*
Catfish	4.9 (MH-4)
Menominee	0.4 (MH-1)
Yellow Perch	0.2 (MH-1)
Salmon	8.4 (MH-1,3)
Walleye	1.1 (MH-1)
Smelt	0.6 (MH-3)
Whitefish	0.5 (MH-1)

*Refer to geographic area in Figure 2.2-1

SOURCE: 1973 Great Lakes Environmental Contaminant Survey - Data Summary and Analysis, Bureau of Consumer Protection, Michigan (1973).

Table 2.2-12
Mean PCB Concentrations in Michigan Tributaries to Lake Huron in 1971-72 and 1973 (ppb as Aroclor 1254)

River	1971-72 Average	1971-72 Range	1973
Saginaw	1.100	0.450-2.900	0.210[b]
Cass[a]	0.014	<0.010-0.048	<0.010
Flint[a]	0.078	0.010-0.150	<0.010
Shiawassee[a]	0.029	<0.010-0.073	<0.010
Tittabawassee[a]	0.140	<0.022-0.230	<0.010
Au Sable	<0.010	≤0.010-0.010	0.013
Thunder Bay	0.023	<0.010-0.037	<0.010
Cheboygan	0.032	<0.010-0.053	<0.010

[a] Tributaries to Saginaw River

[b] Aroclor 1242

SOURCE: Hesse, J.L., Status Report on Polychlorobiphenyls in Michigan Waters, Michigan Water Resources Commission, (June 1973).

Table 2.2-13
Concentrations of polychlorinated biphenyls (PCB's) in Fish from Saginaw River, December 7, 1971
(ppm; wet weight)

Species	Location	Length	Percent fat	PCB's based upon 1242 standard
Perch	Karn Weadock Discharge Channel	9.0	1.3	16.3
Carp	" " " "	21.0	0.9	8.8
Catfish	" " " "	8.0	7.0	37.1
Catfish	" " " "	7.5	4.9	47.9
Carp	U.S. Coast Guard Station	23.0	—	48.0
Carp	" " " "	24.0	10.5	20.4
Carp	" " " "	20.0	6.5	15.5
Carp	" " " "	24.0	5.9	45.8
Carp	" " " "	21.0	4.6	30.2
Pike	" " " "	27.0	1.6	16.6
Pike	" " " "	23.0	1.1	6.9
Gizzard Shad	" " " "	14.0	17.5	165.3
Gizzard Shad*	" " " "	6.0	15.3	32.5
Gizzard Shad*	" " " "	4.5	15.0	161.9
Gizzard Shad*	" " " "	5.0	17.0	77.0
Gizzard Shad*	Zilwaukee Bridge	4.5	15.7	24.1
Gizzard Shad*	" "	5.0	9.6	52.0
Gizzard Shad*	" "	5.0	8.7	10.4

*Concentration based upon analysis of whole fish. All others based upon edible portion only.

SOURCE: Hesse, J.L., Status Report on Polychlorobiphenyls in Michigan Waters, Michigan Water Resources Commission, (June 1973).

2.2.6 Cayuga Lake

In October 1970 lake trout were collected from Cayuga Lake in Ithaca, New York. Since these fish are marked each year when they are stocked in the lake their ages were accurately known. Table 2.2-14 shows the residues of PCB's identified along with the age, length, weight and sex of each fish. The peak heights of individual PCB isomers did not vary with the age of the fish indicating that there is no selective metabolism or storage of specific PCB isomers as the fish matures. However, the concentration of total PCB's does increase progressively with maturity. The variation in PCB concentrations among individual 11 or 12 year old fish may be due to differences among foraging, metabolic and excretary capabilities of the older fish.[1]

Table 2.2-14
Residues of PCB's in Cayuga Lake Trout as a function of maturity; j, juvenile.

Age (years)	Sex	Length (cm)	Weight (g)	PCB (ppm)
1	J			0.6
1	J			1.6
1	J			0.5
1	J			1.2
2	J	27.7	181	2.0
2	J	28.7	226	1.3
2	J	33.5	407	2.5
3	J	44.5	815	2.2
3	J	44.5	725	2.4
3	J	41.1	770	1.2
4	J	53.8	1310	3.5
4	J	50.3	1160	4.1
4	J	55.1	1359	5.1
5	M	61.0	2030	5.7
6	M	63.5	2440	3.4
6	M	66.4	2850	9.7
6	F	68.3	2310	8.6
7	M	63.5	2260	4.0
7	M	68.9	3300	5.5
7		59.7	1990	10.5
8	F	75.2	3390	17.5
8	M	71.6	2805	13.4
8	F	69.0	3300	4.5
9	F	71.2	3390	30.4
11	M	80.3	4200	12.4
12	M	71.6	2535	13.4
12	M	75.5	3120	26.2
12	F	70.6	3440	7.4

SOURCE: Bache, C.A., F.W. Serum, W.D. Youngs and D.J. Lisk, Science, 177, 1191-1192, (1972).

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2.3 Data from Localized Monitoring Efforts - Rivers

2.3.1 Iowa - Mississippi River

Monitoring Iowa rivers for pesticides concentrations over a period of years indicated that chlorinated hydrocarbon insecticides used in row crop agriculture were being carried into the rivers by soil erosion. Since fish are excellent biological compositors, measurement of trace elements in their tissues can give a picture of the relative pollution of a river. Therefore a study of the pesticide levels in the eggs of some fish from locations in Iowa was undertaken in the spring of 1971.[2]

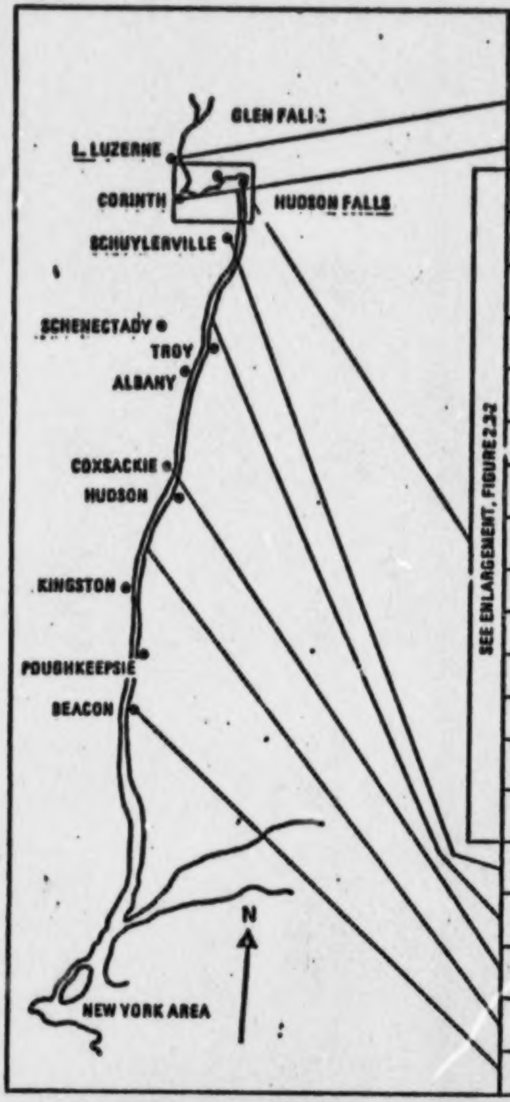
Two of the sites had measurable amounts of polychlorinated biphenyls. The PCB's detected match Aroclor 1254. The concentrations identified in the roe removed from the fish from these sites on the Mississippi were as follows:

Site	Species	Date Collected	Length of Fish	PCB Concentration in 15 grams of roe (ppm)
Near Northern border	Channel Catfish	06-04-71	23.4	1.5
	Largemouth bass	05-20-71	16.4	3.2
	Walleye	04-16-71	27.5	4.2
Near Southern border	Channel Catfish	04-16-71	24.0	0.9
	Largemouth bass	04-15-71	17.5	2.9
	Walleye	04-13-71	24.3	5.5

The levels of PCB's found in the same species from the two locations are approximately the same indicating that the major source of these industrial chemicals is upstream from both of these sites.

2.3.2 New York - Hudson River

The Hudson River in New York State provides an interesting ecosystem in which to study PCB's, primarily because it is the receiving water for effluents from two plants of the General Electric Co., at Hudson Falls and Fort Edward. These plants use PCB's in the production of transformers and capacitors. Summaries of extensive sampling during 1975 of the Hudson River are presented in Figures 2.3-1 and 2.3-2. The GE plants appear to be the major contributors to the PCB residue levels in the river. Although trace levels of PCB's are evident above the GE plant outfall location, levels in water and sediment at Station 1 located at the outfall of the Fort Edward plant were as high as any levels measured in the country. In the immediate area downstream from this outfall, PCB concentrations in water exceed the equilibrium solubility level by more than a factor of 10. Major effects on the levels in sediment are noted more than 35 miles downstream from the outfalls. The data indicate elevated concentrations in aquatic life far from the outfalls: fish samples approximately 40 and 66 miles downstream from the outfalls showed PCB levels in excess of 5 ppm while the same species showed only traces of PCB's directly upstream from the outfalls. Since sediment samples above the plants also show some concentration there does appear to be some PCB source upstream of the plants.



MILE POINT	DESCRIPTION	ESTIMATED PCB'S WATER (PPB)	SEDIMENT (PPM-DRY)	SMALL MOUTH* BASS (PPM)	WALLEYE* (PPM)	WHITE* SUCKER (PPM)	YELLOW* PERCH (PPM)	SNAILS (PPM)	WHITE* PERCH (PPM)
223.0	AT L. LUZERNE		0						
218.0	ABOVE CORINTH	< 0.1	2.4	TRACE		TRACE	TRACE		
214.0	BELOW CORINTH		1.9						
209.0	ABOVE SHERMAN ISLAND DAM		4.0 C						
205.7	ABOVE GLEN FALLS LANDFILL		0.83	TRACE	TRACE	TRACE	TRACE		
204.0	BELOW GLEN FALLS LANDFILL		16.0						
198.0	A* PORTLAND CEMENT CO.		<0.38						
	STATION 0	<1.	0.0				17.0	1.0	
197.3	ABOVE BAKER FALLS DAM		4.0						
196.2	BELOW BAKER FALLS DAM		114. C						
	STATION 1	2800	6700.					45	
	STATION 2	2.5	540.0						
	STATION 3	3.1	2980.0						
	STATION 4	<1	0.6					27.	
194.2	AT FT. EDWARD		147.0						
188.4	ABOVE THOMPSON ISLAND DAM		3707.2 C	122.0**	32.4**	41.0			
183.5	ABOVE SCHUYLERVILLE		78.6						
157.6	ABOVE WATERFORD		19	40.3	32.4	37.6			
124.5	NEAR COXSACKIE	< 0.5		13.76**			4.10		10.7
	ABOVE KINGSTON						6.20		7.30
60.8	NEAR BEACON	< 0.1							

C - MAXIMUM VALUE IN A CORE SAMPLE
* - VALUES ARE WEIGHTED AVERAGES OF SAMPLES OF FISH FROM 3 SIZE GROUPS (EXCEPT AS NOTED) TAKEN AT VARIOUS TIMES IN 1975
** ONLY ONE FISH SAMPLED

SOURCES:
STATIONS 0-4: NADEAU, R.J. AND R.P. DAVIS, INVESTIGATION OF POLYCHLORINATED BIPHENYLS IN THE HUDSON RIVER, 1975, EPA REGION II
OTHER FISH DATA: NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION, DIVISION OF FISH AND WILDLIFE, 1975, ANALYSES OF PCB's IN FISH FLESH
OTHER WATER DATA: NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION, DIVISION OF PURE WATERS, PCB MONITORING IN THE UPPER HUDSON RIVER BASIN, OCTOBER 1975

FIGURE 2.3.-1 MAP OF HUDSON RIVER, NEW YORK, SHOWING DEVELOPED AREAS AND SAMPLING STATIONS WITH PCB LEVELS

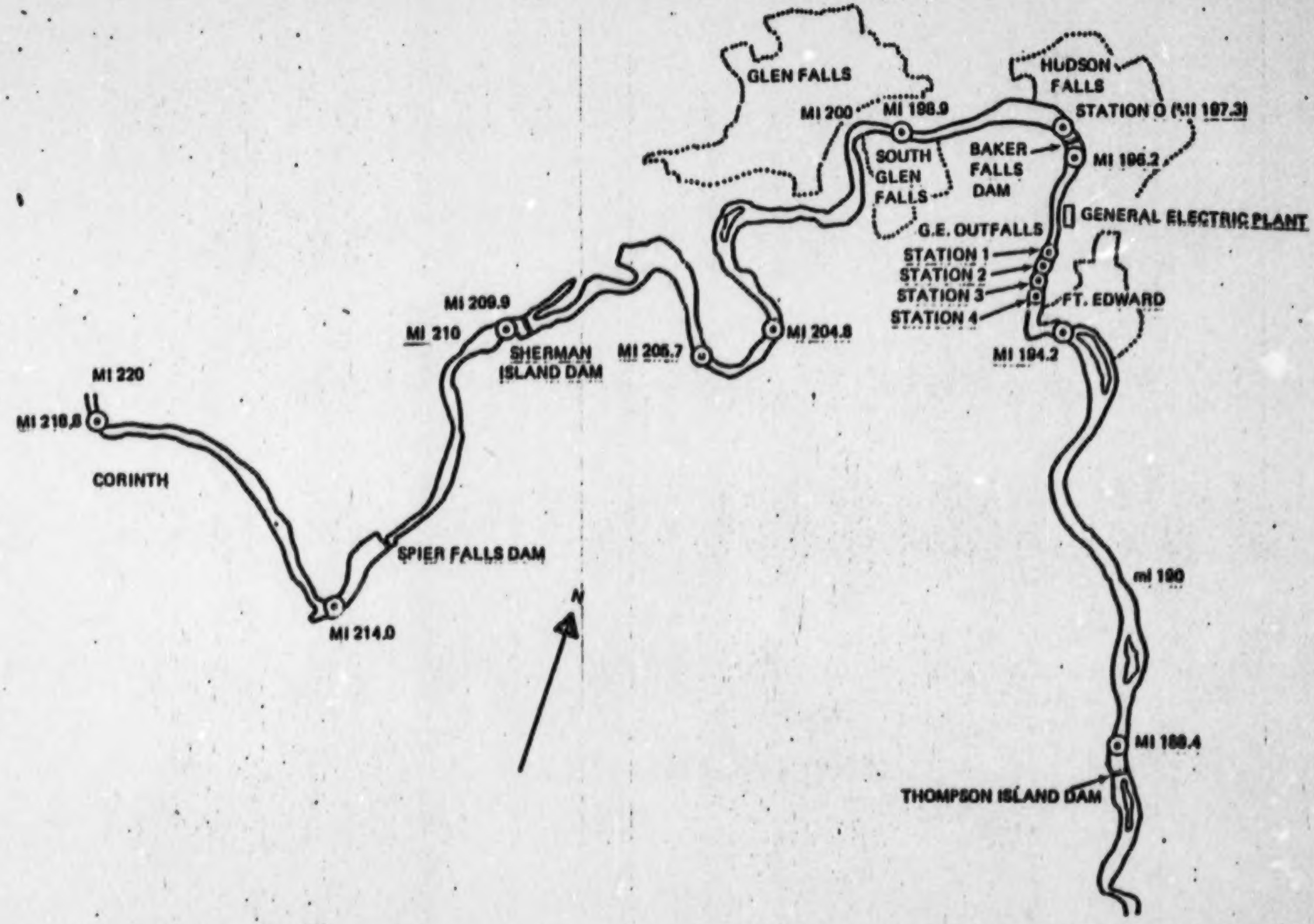


FIGURE 2.3-2 DETAILED MAP OF A PORTION OF THE HUDSON RIVER

2.3.3 Maryland - Chester River

This study was conducted in the Upper Chesapeake Bay in 1971-1972;[1] the study area is shown in Figure 2.3-3 . The Bay and its tributaries receive chlorinated hydrocarbons as a result of industrial, agricultural and waste disposal activities occurring along the shorelines of these waterways and within the total watershed.

Sediment samples were collected approximately quarterly except for the last set which was taken early in order to collect data shortly after tropical storm "Agnes". The PCB's identified were almost exclusively Aroclor 1242. The first readings, taken in November 1971 ranged from 0-21 ppb with an average of 83 ppb. By April 1972 the average had climbed to 110 ppb with a range from 0-300 ppb. By June the readings taken ranged from 0-150 ppb with the average reduced to 53 ppb. After "Agnes" in July the average had again increased to 96 ppb with readings ranging from 0-180 ppb.

The explanation given for the variations is that during the early spring, massive amounts of suspended sediment from the Susquehanna River entered the Chester River and were carried upstream in the flow of water along the river bottom. The peak sediment loads from the Susquehanna generally come at the end of February, decreasing abruptly after the end of March due to a reduced flow rate of the river. An equilibrium net transport develops in the Chester River in April. In May, fine sediments were transported slowly into the Bay. However, over the year there is a movement of sediment along the river bottom into the river from the Bay. Thus, the upper Chesapeake Bay, i.e., the Susquehanna River is the major source of suspended sediments in the lower tidal portion of the Chester River.

The average values and ranges identified in biota of the Chester River taking into account variability due to seasonal fluctuations, distributional differences resulting from sample location, and species as well as individual uptake differences were as follows:

	Average (ppb)	Range (ppb)
Oysters	55	16-250
Soft shelled clams	58	13-180
Fish	18.5	2-570
Crabs	20	0.4-51

Based on data collected June 6, 1972 from the nine stations along the main river course it was determined that concentrations of PCB's decreased as a function of distance up river from its mouth at Love Point in its upstream direction at the rate of 4.20 ppb/Nautical mile.

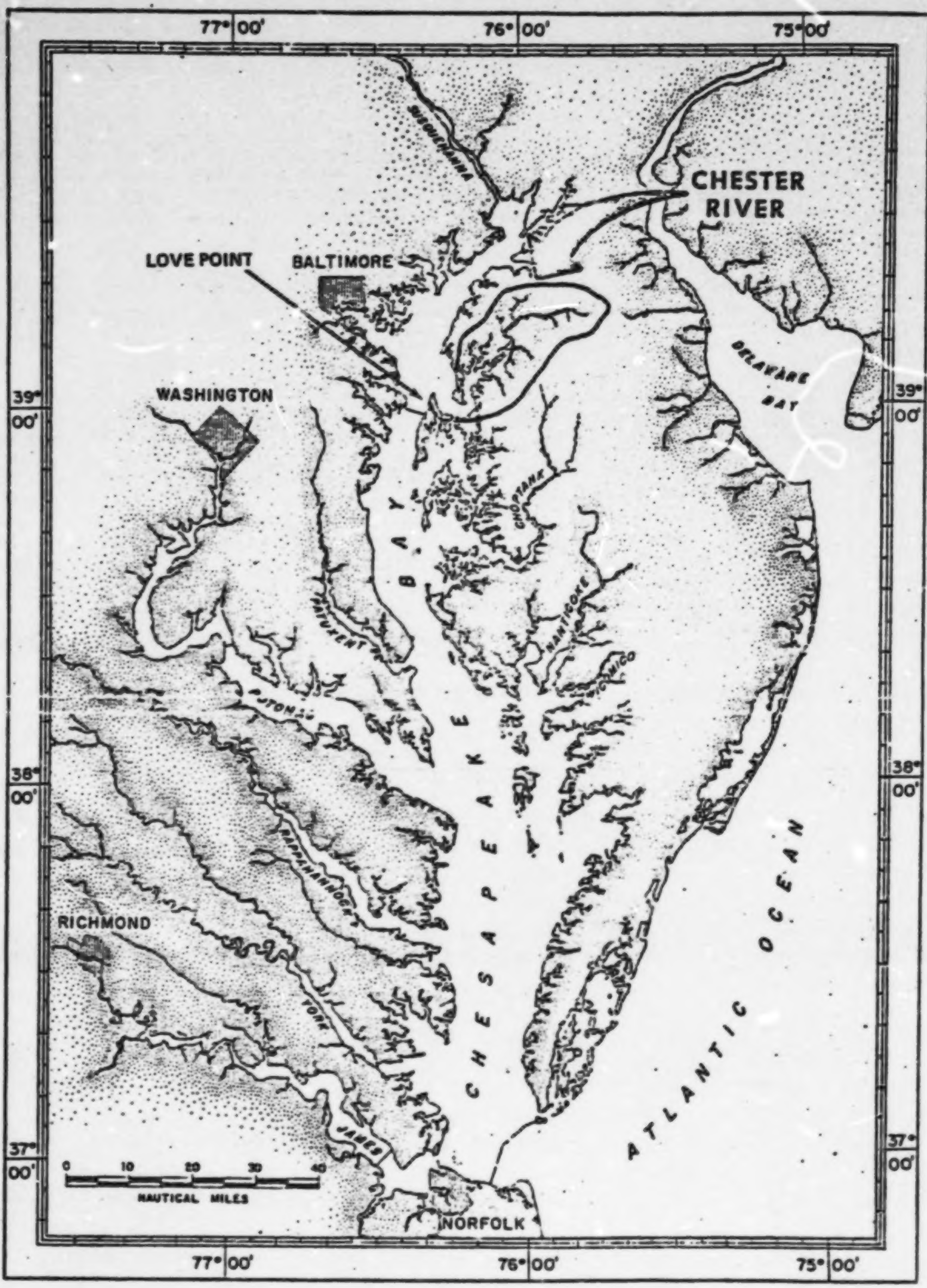


FIGURE 2.3-3 CHART OF CHESAPEAKE BAY SHOWING MAJOR TRIBUTARIES AND LOCATION OF THE CHESTER RIVER.

2.3.4 Connecticut

In October 1974 the State of Connecticut intensively sampled bottom sediment in rivers and lakes thoughout the state. The results are shown in Figure 2.3-4, based on a computer mapping provided by the Connecticut District Office of the U.S. Geological Survey.[6] The data show PCB contamination to be both widespread and at high levels throughout the state, particularly around the highly developed areas of Hartford, New Haven, Stanford-Greenwich and all along the Housatonic River flowing from Massachusetts. The state had sampled mostly whole water for PCB's prior to 1974, with no appreciable residue levels discovered, but now has switched to a yearly bed material determination. The readings for the baseline period of October 1974 are to be updated yearly.

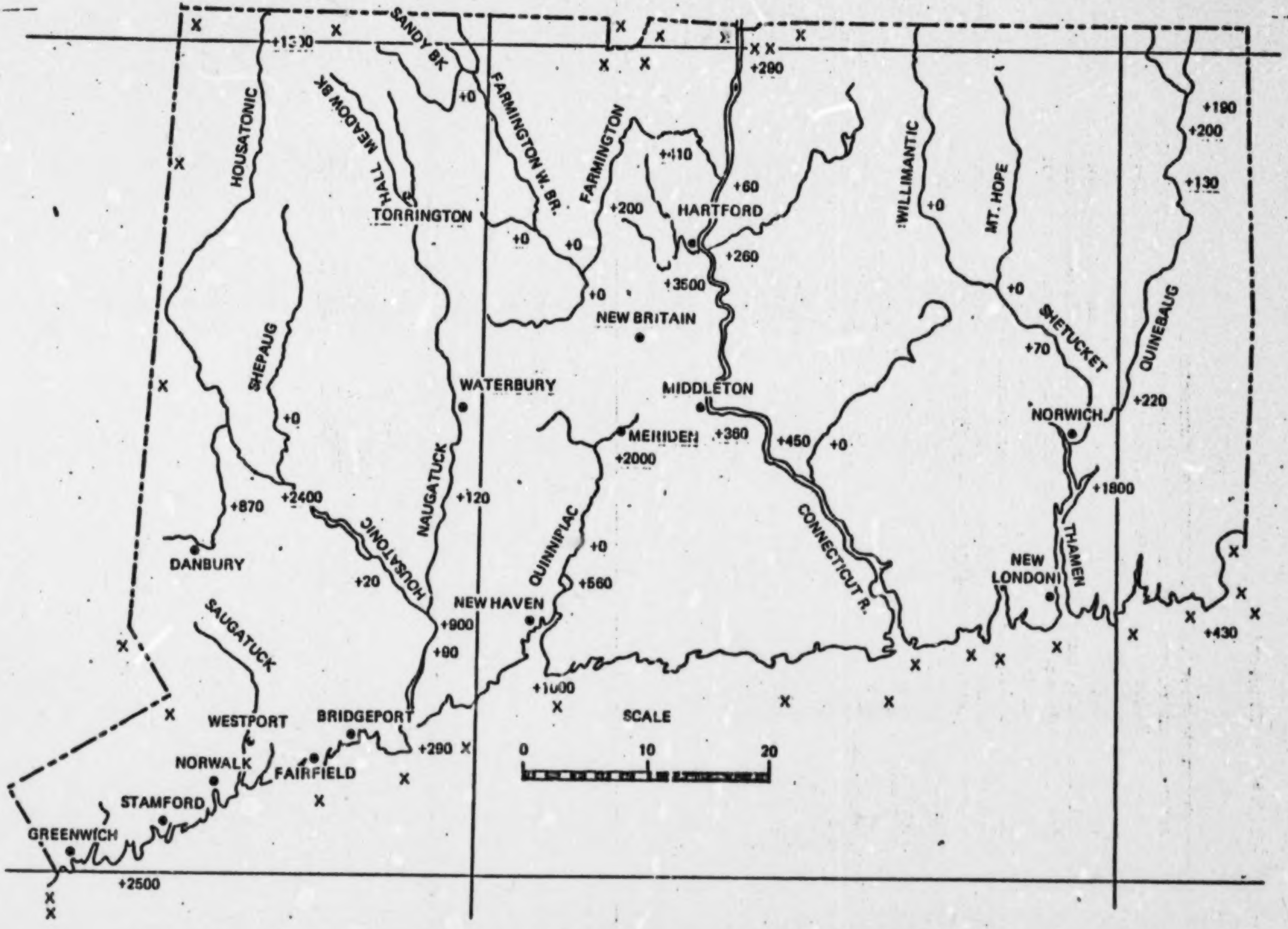


FIGURE 2.3-4 PCB CONCENTRATIONS IN BOTTOM DEPOSITS
STATE OF CONNECTICUT, OCTOBER 1974 (ppb X 10)

REFERENCES, SECTION 2.3

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2.4 Data from Localized Monitoring Efforts - Marine Environment

2.4.1 Atlantic Ocean

In addition to the many PCB studies that have been conducted within the United States, a few studies have been carried out investigating the air, water and fish off the east coast of the United States. These studies, conducted between 1971 and 1974 covering Providence, Rhode Island; Grand Banks; Vineyard Sound; Georges Bank and Bermuda show PCB's to be extensively distributed over the Atlantic Ocean. The general sampling area is shown in Figure 2.4-1.

One of the few air studies that has been published was carried out by Harvey and Steinhauer[11] in 1973. Table 2.4-1 shows the sampling locations and Aroclor 1254 concentrations identified. The highest concentrations were in Vineyard Sound which is approximately 100-150 miles from the Boston, Hartford, New York, New Jersey complex. Over 1000 miles away at Grand Banks the concentrations were 100 times less than in Vineyard Sound. Figure 2.4-2 shows the relationship of atmospheric PCB levels with distance from these industrial areas. The seaward decrease appears to be exponential.

Later studies on PCB's in surface and subsurface waters were done by Harvey[13]. Although the concentration range across the northern North Atlantic is broad, from 1-150 ppt, the average was about 35 ppt in surface waters and 10 ppt at 200 meters depth. Looking at the lower latitudes it appears that the surface waters of the Sargasso Sea have slightly lower concentrations than other parts of the North Atlantic, averaging 27 ppt. The widespread PCB distribution implicates the atmosphere as a predominant mode of transport in the Atlantic with variations due to the seaslicks, localized rainfall or discharges from ships.

During the period of February-June 1973, PCB concentrations in the marine atmosphere of the Bermuda - Sargasso Sea and Providence, Rhode Island area were measured by Bidleman and Olney[1]. It is interesting to note that most of the PCB was trapped on the polyurethane foam collection surface suggesting that PCB's are in the atmosphere mainly as vapors rather than adsorbed onto particulate matter, or that they volatilize from the trapped particles collected on the glass fiber prefilter. Table 2.4-1 gives the locations and levels identified as Aroclor 1242 or 1248.

Sargasso Sea surface water was also measured by Bidleman and Olney[1] in 1973. From Table 2.4-2 it can be seen that the concentrations were higher in the surface layer than in the subsurface samples.

Follow-up by Harvey[12], et. al., indicated that PCB concentrations in North Atlantic surface waters declined forty fold from 1972 to 1974 presumably following the cessation of certain industrial uses of those compounds. However, Longhurst and Radford dispute the decrease stating that either the analytical methods were inaccurate or Harvey's original estimate of the total amount of dissolved PCB's in the upper 200 m was incorrect. Harvey and Steinhauer have agreed[17] that their extrapolation may well have been improper and planned more sampling for 1975.

In 1971 a seafood monitoring program[18] was conducted on the East Coast of the United States. It is difficult to make interspecies comparisons but as can be seen in Table 2.4-3 the highest Aroclor 1260 level was in flounder from Jacksonville, Florida.

Table 2.4-1
PCB Concentrations Over the Western North Atlantic

Station	Sample volume (m^3)	ng/m^3
Bermuda	560	0.5=
	480	0.4=
	820	0.16=
	500	0.15=
	1070	0.59∝
	1320	0.30∝
	918	0.65∝
	1950	0.62∝
	1740	0.55∝
	732	0.52∝
	1300	0.61∝
	860	0.21∝
33°20'N, 65°14'W	300	1.6∝
34°39'N, 66°15'W	267	0.79∝
38°48'N, 69°14'W	222	0.72∝
40°32'N, 70°20'W	196	0.83∝
Georges Bank (41°40'N, 67°30'W)	105	1.4=
	675	0.82=
	660	0.58=
	655	0.61=
	640	0.80=
	650	1.60=
Vineyard Sound (41°20'N, 70°50'W)	105	3.9=
	224	5.3=
Grand Banks (45°16'N, 52°08'W)	780	0.05=
	960	0.07=
	840	0.10=
	940	0.16=
	540	0.05=
Providence, R.I.	392	4.0∝
	1071	2.1∝
	744	5.8∝
	76	9.4∝

=Calculated as Aroclor 1254
∝Calculated as Aroclor 1242 or 1248

SOURCE: Harvey, R.G. and W.G. Steinhauer, Atmospheric Environment, Nature, 252, 387-388 (November 29, 1974).

Table 2.4-2
PCB's Measured in Sargasso Sea Area, 1973

Location	Sample*	ppt**
29°56'N, 64°40'W	SM	11.2
	SS	3.6
30°45'N, 66°55'W	SM	4.9
	SS	<0.9
30°34'N, 66°59'W	SM	8.3
	SS	1.0
28°53'N, 65°07'W	SM	42.6 , 19.3
	SS	<0.9 , <0.9
29°56'N, 63°00'W	SM	3.8
	SS	<0.9
30°00'N, 64°30'W	SM	5.6
	SS	1.6
31°34'N, 63°49'W	SM	5.0
	SS	1.8
31°38'N, 63°57'W	SM	8.4
	SS	0.9

*SM - sample is surface microlayer
SS - sample is subsurface water

**PCB calculated as Aroclor 1260

SOURCE: Bidleman, T.F., and C.E. Olney, Science, 184, 516-518 (Feb. 8, 1974).

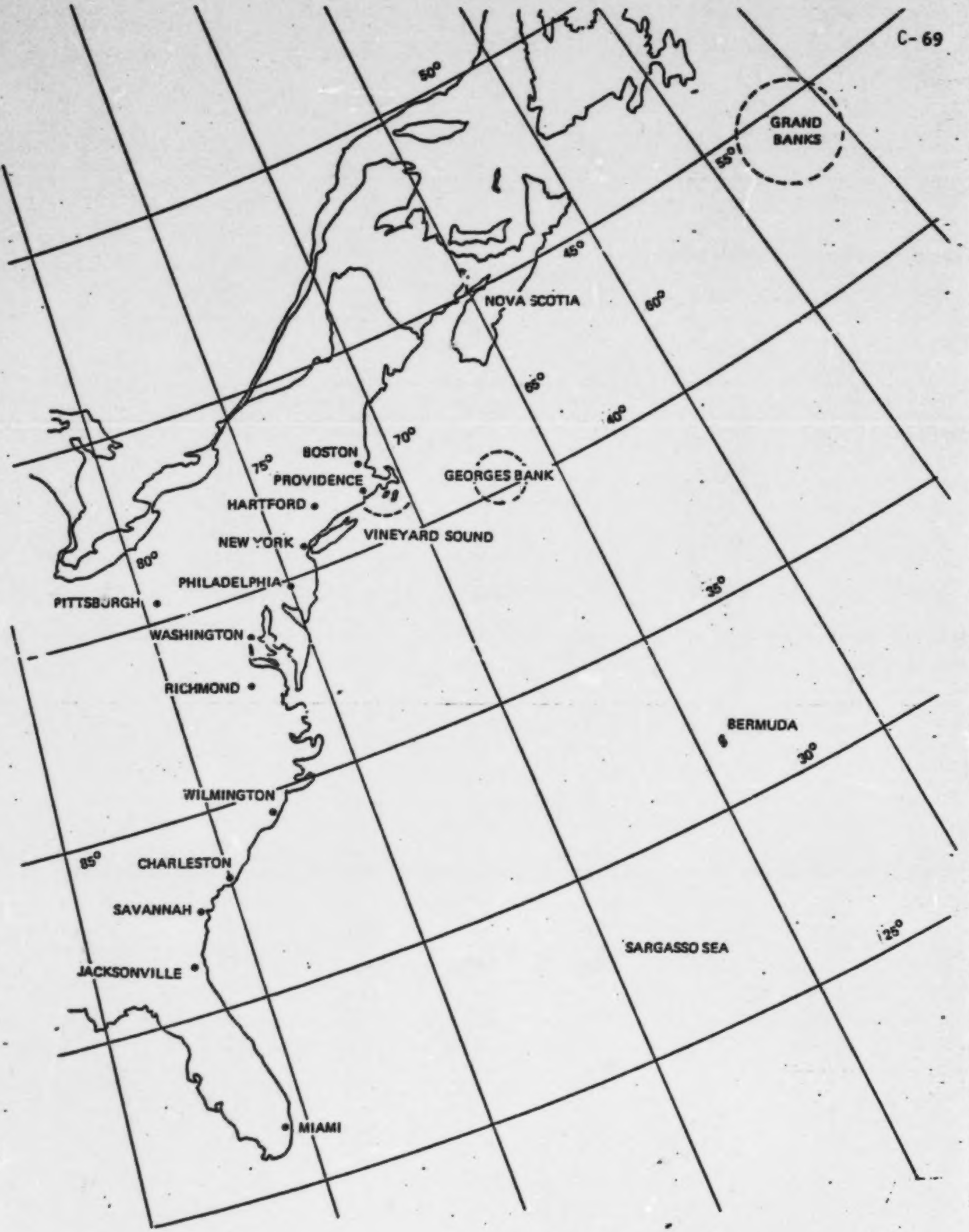
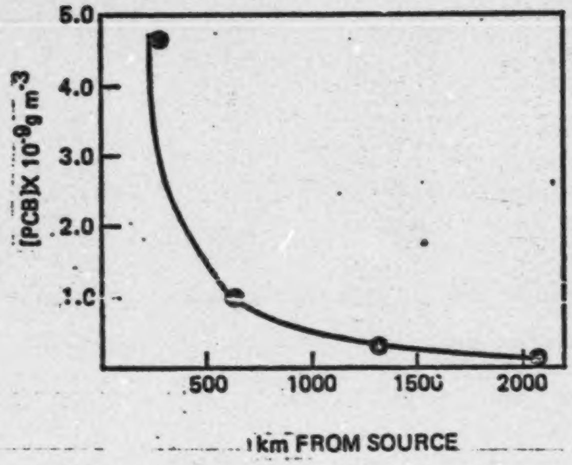


FIGURE 2.4-1 ATLANTIC OCEAN SAMPLING SITES



SOURCE: HARVEY, G.R. AND W.G. STEINHAUER, ATMOSPHERIC ENVIRONMENT, 8, 777–782, (1974).

FIGURE 2.4-2 RELATIONSHIP OF ATMOSPHERIC PCB LEVELS WITH DISTANCE FROM INDUSTRIAL AREAS

Table 2.4-3
PCB Residues in Seafood

Species	Sample Quantity	Aroclor 1260 (ppm)
	JACKSONVILLE, FLA.	
Blue crabs	15	0.08
Shrimp	1 kg	0.60
Flounder	3	0.65
Weak fish	5	0.44
Drum	5	0.17
	SAVANNAH, GA.	
Blue crabs	16	—
Shrimp	0.5 kg	—
Pilot shrimp	0.5 kg	—
Oysters	15	—
Razorback clams	28	—
Squid	5	—
Flounder	4	—
	CHARLESTON, S.C.	
Blue crabs	10	—
Shrimp	1 kg	0.02
Oysters	>15	—
Squid	>15	—
Croaker	1	—
	MOREHEAD CITY, N.C.	
Blue crabs	15	—
Shrimp	1 kg	—
Oysters	24	0.02
Clams	20	—
Scallops	12	—
Flounder	3	—
Spanish mackerel	2	—
Weak fish	2	—
Blue fish	2	—
Fish meal	0.5 kg	0.11
	CHESAPEAKE BAY	
Blue crabs	25	0.02
Weak fish	3	0.49
Striped sea bass	3	0.02
Spot	5	0.10
Herring	10	—
	DELAWARE BAY	
Blue crabs	15	0.13
Flounder	2	0.07
Weak fish	5	0.23
Striped bass	2	0.07
Mullet	5	0.21
Croaker	1	0.50

SOURCE: Markin, G.P., J.C. Hawthorne, J.L. Collins and J.H. Ford Pesticides Monitoring Journal, 7, 139-143, (March 1974).

2.4.2 Bay of Fundy

As early as 1969, PCB residues were identified in harbour porpoises from the Bay of Fundy, located in the northeast section of Maine[5]. Zitko, et. al.[24], identified Aroclor 1254 in a number of different fish ranging from 0.07 ppm in the muscle of basking shark and 0.21 ppm in the muscle of sea raven to 3.55 ppm in Herring Oil and 218 ppm in the liver of white shark. The species studied and the levels measured are listed in Table 2.4-4.

Table 2.4-4
PCB and Chlorinated Hydrocarbon Pesticides in Aquatic Animals from the Bay of Fundy - Gulf of Maine Area.

Species	Tissue	Aroclor 1254, ppm
Herring	whole fish	0.34
Mackerel	muscle	0.35
Plaice	muscle	0.38
White hake	muscle	0.44
Ocean perch	muscle	0.32
Cod	muscle	0.55
Sea raven	muscle	0.21
	viscera	0.73
Basking shark	muscle	0.07
	liver	1.07
White shark	muscle	0.77
	liver	218
Bluefin tuna	muscle	1.54
Herring oil		3.55
Fishmeal		0.54
Double-crested cormorant	eggs	43.5
		17.2
	muscle	3.38
	liver	2.13
	subcutaneous fat	38
	abdominal fat	52
Herring gull	eggs	12.6
		5.54
	muscle liver	5.06
		6.50
	subcutaneous fat	75

SOURCE: Zitko, V., O. Hutzinger and P.M.K. Choi, Environmental Health Perspectives, 47-50, (April 1972).

2.4.3 Gulf of Mexico - Caribbean Sea

Runoff from approximately two-thirds of the United States and one-half of Mexico enters the Gulf of Mexico and is swept generally westward to be trapped in the Western Gulf for as much as 100 years. Therefore a buildup of man-made toxic chemicals is possible in the Western Gulf especially since there is a heavy concentration of chemical manufacturers in Louisiana and Texas.[7]

The earliest samples collected in this area were brown Pelicans collected in 1969 from the Atlantic and Gulf Coast of Florida and in 1970 from Florida Bay. The highest levels, 1.0-7.5 ppm came from the Atlantic Coast while those from the Gulf Coast ranged up to 4.2 ppm and those from the Keys in the Bay only had concentrations up to 2.5 ppm. The birds represented a wide range of ages and both sexes, but the immature and adult birds always had higher residues than the young.[2]

In 1971 a seafood monitoring program[18] for the insecticide mirex was set up. Three of the stations were located in the Gulf of Mexico and identified Aroclor 1260 as indicated in Table 2.4-5. Due to the different kinds of species involved a comparison of levels is difficult to prepare. However, it is clear that the levels in samples from Mobile Bay, Alabama ranging from 0.01 to 0.40 ppm were higher than those from the Mississippi Sound where the highest level was only 0.20 ppm.

Fish and other marine organisms were collected in the Gulf of Mexico and the Caribbean Sea in May and October 1971 as shown in Figure 2.4-3. While PCB's were detected in nearly all the samples analyzed the levels were generally low. The samples from coastal waters generally had higher levels than samples from open waters. This same observation holds true for sea plankton. Of the six samples containing PCB's above 100 ppb wet weight four of them were near Coastal areas.[9]

Groupers were also collected[8] since they tend to spend their post larval life in one locality and should therefore yield data more representative of a particular area. Figure 2.4-3 shows the sampling locations. The levels are generally low although the highest levels do come from the western Gulf. Note also that at Anton Lizardo the levels in the groupers increase with increasing size as can be expected since the fat content of the fish increases with increasing size and age of the specimen. Table 2.4-6 presents some of the levels identified in 1971.

Giam et. al.[6] again took measurements from stations in the Gulf of Mexico in 1973-1974 as indicated in Figure 2.4-3 and Table 2.4-7. The concentrations in biota are lower than they were in the 1971 survey with an average according to Giam of about 20 ppb reduced from 60 ppb. It must be noted, however, that these stations were all close to the southern coast of the U.S. with no samples collected in the Western Gulf.

Table 2.4-6
Biota and Plankton from the Gulf of Mexico - 1971

Station	Biota	Weight (lbs.)	Standard Length (mm)	PCB's (ppb)
1	Flounder∞			32
2	Flounder∞			36
3	Flounder∞			59
4	Flounder∞			34
	Plankton†			678
5	Crustacean∞			151
	Crustacean∞			22
6	Shrimp∞			167
7	Rock Shrimp∞			6
	Squid∞			40
8	King Mackerel muscle∞			34
	Plankton†			<3
	Tuna muscle∞			58
9	Tuna muscle∞			36
10	Plankton†			100
11	Plankton†			30
12	Plankton†			<3
13	Plankton†			1055
14	Plankton†			44
15	Plankton†			42
16	Plankton†			191
27	GrouperΩ	3.1	398	110
		2.9	378	32
		1.9	306	14
		1.9	287	14
		0.7	222	12
28	GrouperΩ	4.9	414	33
		4.8	416	12
		2.8	338	10
29	GrouperΩ	10.0	514	7
		3.7	403	7
		2.9	354	6
30	GrouperΩ	6.0	495	5
		4.8	459	3
		1.6	315	3
		3.0	385	14
		2.4	339	6
31	GrouperΩ	3.3	352	81
32	GrouperΩ	18.0	681	220

∞Giam, C.S., A.R. Hanks, R.L. Richardson, W.M. Sackett and M.K. Wong, Pesticides Monitoring Journal, 6, 139-143, (Dec. 1972).

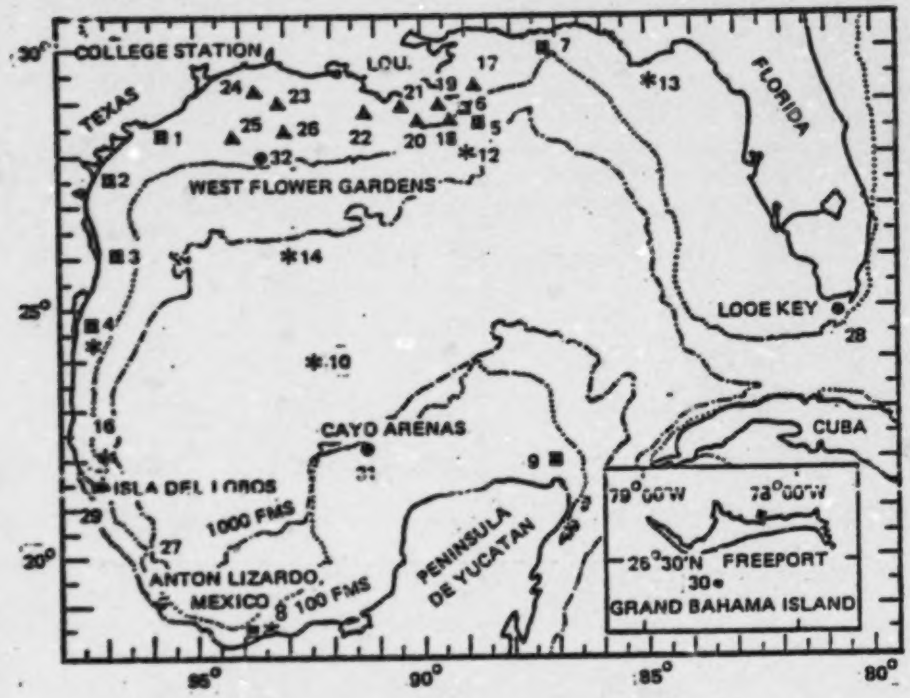
†Giam, C.S., M.K. Wong, A.R. Hanks, W.M. Sackett and R.L. Richardson, Bulletin of Environmental Contamination and Toxicology, 9, 376-382, (1973).

ΩGiam, C.S., R.L. Richardson, D. Taylor and M.K. Wong, Bulletin of Environmental Contamination and Toxicology, 11, 189-192, (1974).

Table 2.4-5
PCB Residues in Seafood

Species	Sample Quantity	Aroclor 1260 (ppm)
	MISSISSIPPI SOUND	
Crabs	15	0.07
Shrimp	1 kg	——
Squid	1 kg	0.03
Flounder	3	0.03
Speckled sea trout	3	0.20
Spanish mackerel	3	0.06
Weak fish	5	0.12
Atlantic whiting	5	0.10
Fish oil	1 liter	0.02
Fish meal	1 kg	0.02
Ground fish (unprocessed)	1 kg	0.04
	MOBILE BAY, ALA.	
Blue crabs	15	0.12
Shrimp	1.3 kg	0.01
Oysters	20+	0.03
Squid	10	0.04
Flounder	3	0.11
Speckled sea trout	1	0.40
Spanish mackerel	2	0.18
Weak fish	5	0.37
Atlantic whiting	5	0.11
Croaker	3	0.13
Red snapper	2	0.14
Mullet	5	0.11
Anchovy	>20	0.08
Shad	>10	0.09
	TAMPA BAY, FLA.	
Crabs	10	0.03
Weak fish	5	0.83
Mullet	5	0.31

SOURCE: Markin, G.F., J.C. Hawthorne, J.L. Collins, and J.H. Ford, Pesticides Monitoring Journal, 7, 139-143, (March 1974).



■ BIOTA 1971

● GROUPERS 1971

▲ BIOTA, WATER, SEDIMENT, 1973–1974

✱ PLANKTON 1971

FIGURE 2.4-3 GULF OF MEXICO SAMPLING SITES

Table 2.4-7
Concentrations of PCBs in Selected Samples from the Gulf of Mexico, 1973-74

Station	Biota (ppb)	Water (ppt)	Sediment (ppb)
7	20	4.1	<0.2
18	11	2.1	35.0
19	22	1.7	33.0
20	23	——	<0.2
21	37	1.7	<0.2
22	14	2.1	——
23	6	2.4	——
24	11	1.3	——
25	94	0.8	<0.2
26	68	2.7	——

SOURCE: Giam, C.S., J.S. Chan, J.P. Kakareka and G.S. Neff, Trace Analyses of Phthalates and Chlorinated Hydrocarbons in Gulf of Mexico Samples.

2.4.4 California

According to Hom and Risebrough[14] PCBs have become a significant componant of the marine food webs of southern California. They have been associated with a high incidence of premature births among sea lions and eggshell thinning with consequent reproductive failure in fish-eating birds.

One study collected different levels of sediment from the Santa Barbara Basin.[14] The dated and analyzed sediments indicated that the deposition of PCB's began about 1945 probably as a result of the rapid increase in PCB use as electrical insulating fluids and paint additives during World War II. Through 1967 there was no indication of a leveling off in the rate of PCB deposition. Levels in the 1940-1945 layer were 31 ppb increasing to 49 ppb in the 1947-1952 layer, 66 ppb in the 1955-1960 layer and 103 ppb in the 1962-1967 layer.[14]

Risebrough[23] has suggested that the observation of PCB's in the sea indicate that they are dispersed by wind currents. Table 2.4-8 shows the distribution of PCB residues in several collections of marine fish collected from the Coastal waters of southern California in late 1965 and early 1966 and in marine birds collected in late 1966. Note that the levels in the birds 0.08-109 ppm were higher than those in fish, N.D.-1.2 ppm. Petrels and shearwaters breed on remote islands spending their entire lives at sea. They do not dive for fish but feed primarily upon organisms obtained at or near the surface where aerial fallout could be expected to retain temporaily the water-insoluble chlorinated hydrocarbons components. Two Peregrine Falcons showed PCB levels ranging from 1.5 ppm, net weight, in the brain of an immature bird to 1,980 ppm lipid weight in the carcass of an adult bird.

Analyses of Western gull eggs[22] show that eggs from San Francisco Bay, with levels from 24-950 ppm contained more PCB's than eggs from the Farallon Islands 27 miles west of the Golden Gate Bridge with levels from 12-1010 ppm. These were both higher than eggs from Baja California where the levels were 1.2-471 ppm.

Concern over reproductive failure of the Double Crested Cormorant led to a study in 1969 of Cormorant eggs from three locations in southern California. The levels identified ranged from 12-1,100 ppm in the yolk lipids.[10] A similar concern also led to a study of Brown Pelicans on Anacapa Island in May 1969. Fat samples from six adult birds and one immature bird ranged from 77 to 366 mg/gm.[15]

Early data collected by Munson[19] in 1970 from San Diego and Orange Counties showed levels in aquatic biota ranging from <2.0 to 8.8 ppm lipid weight (N.D. - 1.0 wet weight) in San Diego and 0.62 - 38 ppm lipid weight (0.008 to 1.4 wet weight) from Orange County. However, the difference in residue levels may be due to a different type of uptake mechanism since the samples from Orange County were invertebrates while those from San Diego were fish.

In addition to the studies of Double Crested Cormorants and Brown Pelicans, Faber et. al.[4] studied Common Egrets and Great Blue Herons. The PCB levels ranged up to 15 ppm in the brain and 93 ppm in the livers of adult egrets from the Audubon Canyon Ranch. Fish which the birds might feed on from Bolinas Lagoon showed levels from 0.072-0.079 ppm.

In order to assess the potential contamination from chlorinated hydrocarbons the U.S. Geological Survey initiated a study in February 1972 of the San Francisco Bay. Bottom material was collected from 26 streams that discharge into San Francisco Bay. The results of the analyses are given in Figure 2.4-4 illustrating the widespread distribution of PCB's in the San Francisco Bay area. The readings from Steven Creek of 180 ppb and from Alamitos Creek, of 610 ppb were higher than anticipated since neither area had any apparent industrial or commercial development. Despite the extreme range up to 1400 ppb there was no significant difference between the average residue of streams discharging into the Bay south of San Francisco and those discharging into the Bay north of San Francisco.[16]

Studies of Waste Water Treatment Plants in the Southern California area will be presented in Section 2.5.3.4.

Table 2.4-8
Polychlorinated Biphenyl (PCB) Residues in Marine Fish and Marine Birds, 1965-1966

Species Locality,	PCB
Northern Anchovy	
Terminal Island	1.0
Shiner Perch	
San Francisco Bay	0.4-1.2
English Sole	
San Francisco Bay	0.05-0.11
Monterey	0.04
Jack Mackerel	
Channel Islands	0.02
Hake	
Puget Sound	0.16
Channel Islands	0.12
Bluefin Tuna	
Body muscle	0.04
Liver	0.04
Yellowfin Tuna	
Liver	0.04
Skipjack Tuna	
Liver	0.1
Cassin's Auklet	0.16
Ancient Murrelet	0.15
Fulmar	0.08
Fulmar	0.34
Red Phalarope	0.10
Rhinoceros Auklet	0.36
Slender-billed Shearwater	2.1
Sooty Shearwater	1.2
Sooty Shearwater	0.9
Peregrine Falcon	
Breast muscle, second year Female, migrant from Artic	22
Breast muscle, immature California	10.5
Breast muscle, adult female, California	109

SOURCE: Risebrough, R.W., Chlorinated Hydrocarbons in Marine Ecosystems.

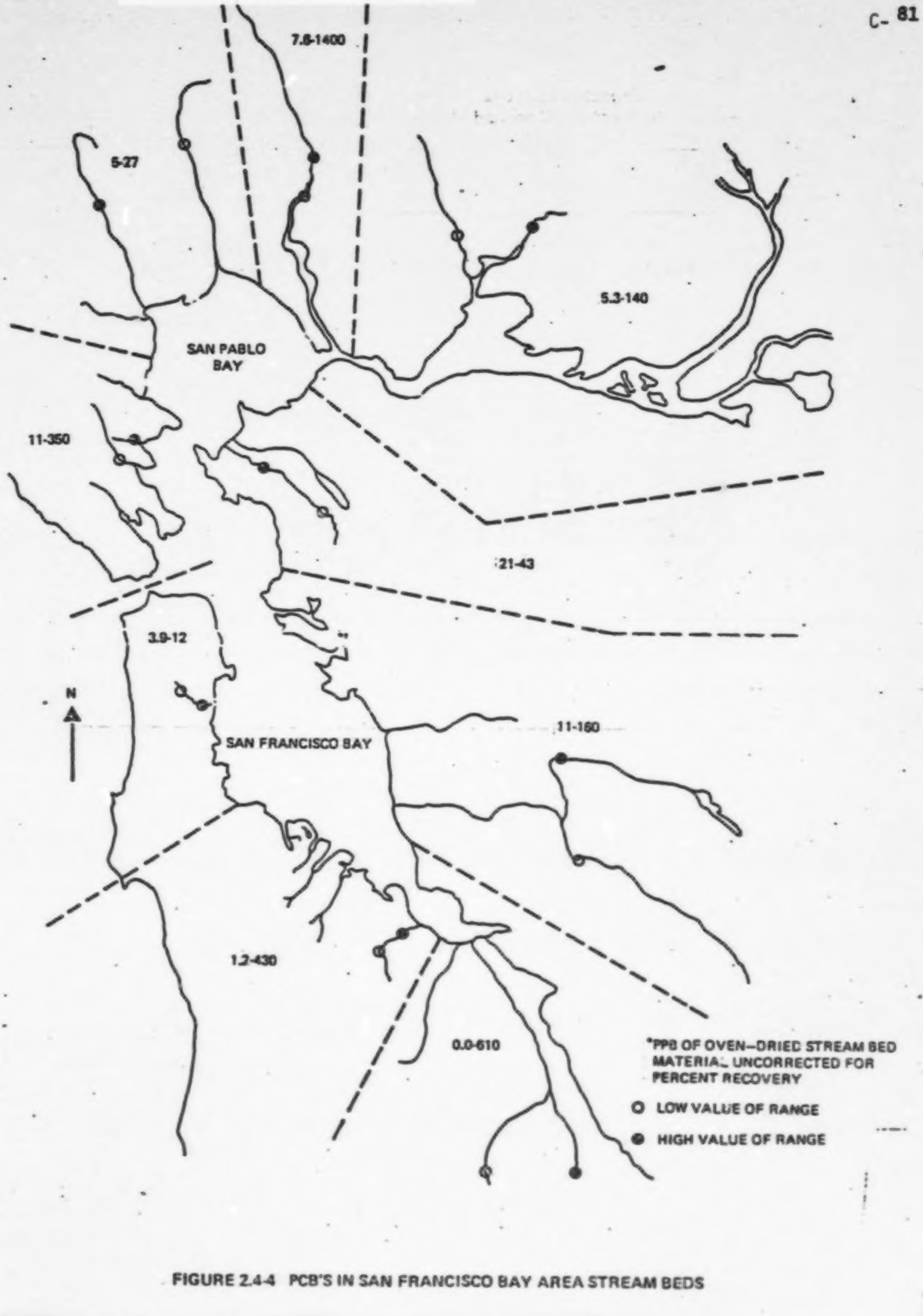


FIGURE 2.4-4 PCB'S IN SAN FRANCISCO BAY AREA STREAM BEDS

2.4.5 Escambia Bay, Florida

The first detection of Aroclor 1254 residues in Escambia Bay was in oysters in April 1969.[3] Later sampling also showed residues in water, sediment, fish, blue crabs and shrimp as indicated in Table 2.4-9. One source was apparently an industrial outfall which had had accidental leakage of a heat exchange fluid. Fish, shrimp and crabs contained higher concentrations than oysters but are also more mobile and therefore not as useful as monitors for a particular area.

Figure 2.4-5 identifies the sampling locations (stations 1-7). Less than 0.1 ppb occurred in the water at station 2 but it was not detected in lower bay water. Leaching from sediments is presumably the cause of the continued presence of Aroclor 1254 in the river water. Sediment samples taken near the outfall reached 486 ppm in August 1969.[3]

Pink shrimp collected at the same time from the bay were found to contain whole body residues of Aroclor 1254 as high as 14 ppm.[20] Residues in seven composite samples of at least five shrimp ranged from 0.6 to 120.0 ppm. Fiddler crabs collected in April 1970 from the lower Escambia River and Upper Escambia Bay had individual whole body residues of 0.45 to 1.5 ppm.

The largest accumulations were found in the sediments with the maximum residue of 61 ppm observed in the River at the outfall from the industry. The maximum in the Bay of 30 ppm was found near the mouth of the river. Although Aroclor was not detected in sediments collected above the plant, soil samples from the bank near the mouth of the river downstream from the source 6.5 km had 1.4 to 1.7 ppm. Subsequent samplings from three stations in the bay showed little change in the chemical even after nine months. Table 2.4-9 shows the levels identified for these sediment collections as stations 8-20.[20]

The amount of PCB in sediment samples appeared to decrease after the initial February 1970 survey.[21] The decrease is especially noticeable in December 1970 and October 1971. In general, the residues identified in 1971 were about one-tenth the 1970 values. Cores taken in a 1972 survey generally indicated less PCB than in 1971. Table 2.4-9 identifies the readings taken for these three years as stations 21-23.

Later surveys of biota from the estuary showed levels to remain relatively high. Table 2.4-10 compares residues in the same species or those occupying similar trophic levels captured on the same day in Escambia and East Bays; the East Bay site being about 35 kilometers from the original source of the material. Although sand seatrout and Atlantic cutlassfish could be expected to have the highest residues since they are predators the highest levels were actually found in silversides whose diet consists mainly of plankton. Note also that the concentrations in species from Escambia Bay were 5 to 10 times greater than those found in East Bay but that Aroclor 1254 was found even in species captured distant from the original source of PCB.[21]

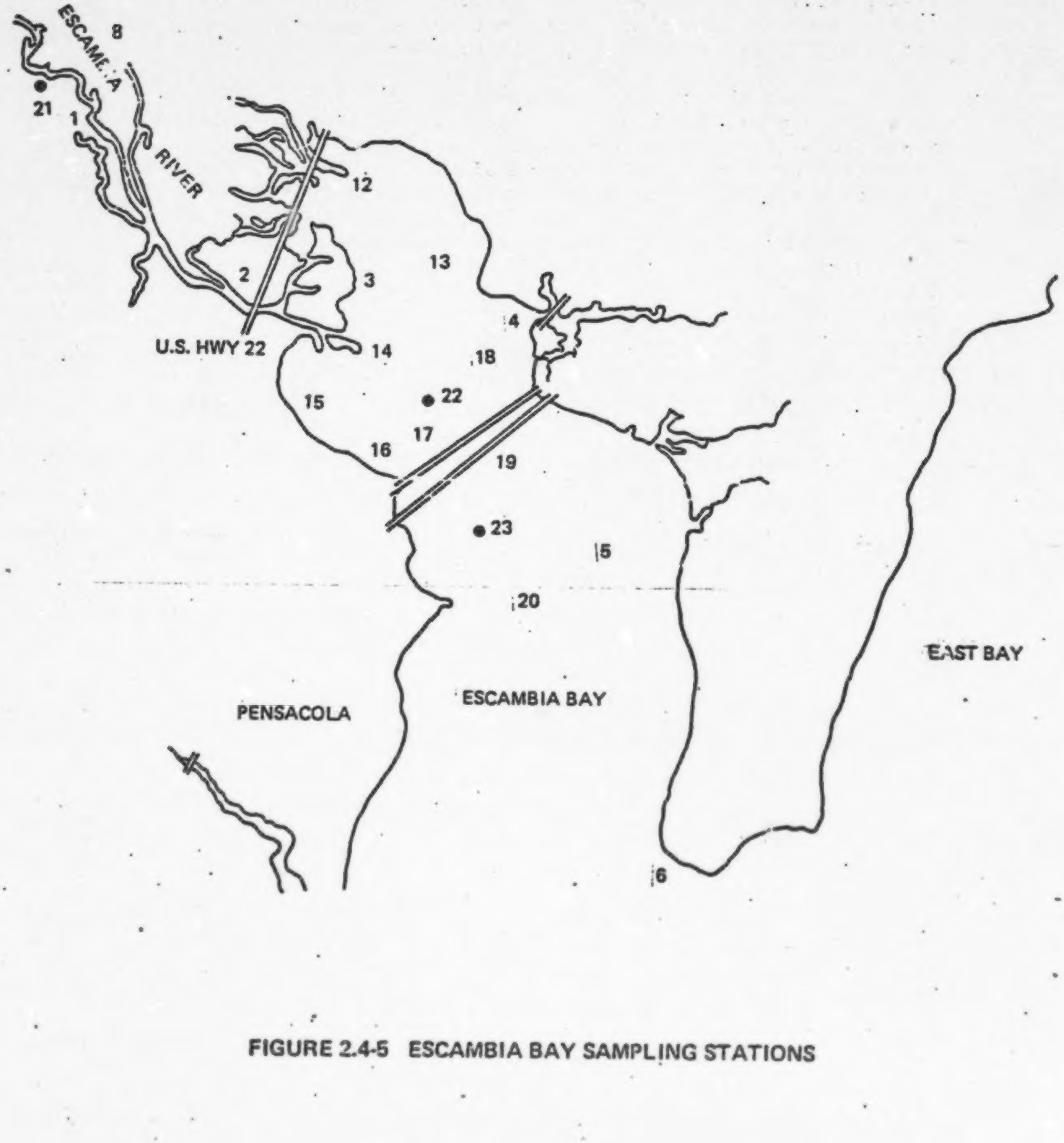


FIGURE 2.4-5 ESCAMBIA BAY SAMPLING STATIONS

Table 2.4-9
Residues of Aroclor 1254 in Samples from Escambia Bay and River

		Residues of Aroclor 1254 (ppm)			
Location	Sample	1969	1970	1971	1972
3	Speckled trout	20.0			
3	Flounder liver	184.0			
4	Menhaden	5.7			
4	Menhaden	11.0			
4	Menhaden	12.0			
4	Flounder liver	76.0			
4	Flounder muscle	4.5			
4	Flounder gills	19.0			
4	Croaker	12.0			
4	Pinfish	10.0			
3	Shrimp	2.5			
3	Blue crab	7.0			
3	Blue crab	6.3			
4	Shrimp	1.5			
4	Blue crab	1.0			
1	Sediment	486			
2	Sediment	<.03			
6	Sediment	1.7			
8	Sediment		N.D.		
9	Sediment		61.0		
10	Sediment		5.7		
11	Sediment		4.1		
12	Sediment		1.9		
13	Sediment		1.8		
14	Sediment		30.0		
15	Sediment		4.2		
16	Sediment		3.3		
17	Sediment		4.9		
18	Sediment		0.6		
19	Sediment		2.5		
20	Sediment		1.4		
21	Sediment 0-2 in.		78.0	8.1	0.97
	Sediment 2-4 in.		30.0	0.12	5.8
	Sediment 4-6 in.		6.1	N.D.	——
	Sediment 6-8 in.		0.4	N.D.	——
22	Sediment 0-2 in.		10.0	0.91	0.14
	Sediment 2-4 in.		11.0	N.D.	N.D.
	Sediment 4-6 in.		15.0	N.D.	N.D.
	Sediment 6-8 in.		20.0	N.D.	——
	Sediment 8-10 in.		16.0	N.D.	——
	Sediment 10-12 in.		1.2	N.D.	——
23	Sediment 0-2 in.		0.19	0.19	——
	Sediment 2-4 in.		0.08	N.D.	——
	Sediment 4-6 in.		0.02	N.D.	——
	Sediment 6-8 in.		N.D.	N.D.	——

SOURCE: Nimmo, D.R., D.J. Hansen, J.A. Couch, N.R. Cooley, P.R. Parrish and J.I. Lowe, Toxicity of Aroclor 1254 and its Physiological Activity in Several Estuarine Organisms, (unpublished).

Table 2.4-10
Comparison of Concentrations of Aroclor 1254 Found in Species Collected in Escambia and East Bays

Species	Escambia Bay (ppm)	East Bay (ppm)
Spartina	N.D.	N.D.
Zostera	N.D.	N.D.
Olive Nerite	0.49	N.D.
Rangia	N.D.	N.D.
Penaeid Shrimp	0.98	Trace
Blue Crabs	6.90	0.46
Bay Anchovy	3.00	0.68
Catfish	3.80	0.58
Tidewater Silversides	10.00	0.95
Silver Perch	4.50	0.48
Sand Seatrout	1.50	——
Spotted Seatrout	——	0.12
Spot	1.80	Trace
Atlantic Croaker	1.60	Trace
Hogchoker	1.30	N.D.
Atlantic Cutlassfish	2.90	0.72

SOURCE: Nimmo, D.R., D.J. Hansen, J.A. Couch, N.R. Cooley, P.R. Parrish and J.I. Lowe, Toxicity of Aroclor 1254 and its Physiological Activity in Several Estuarine Organisms, (unpublished).

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2.5 Data from Localized Monitoring Efforts Industrial Plants, Products, Sewage Treatment Facilities and Landfills

2.5.1 Industrial Plants

.5.1.1 Monsanto Co., Sauget, Illinois

The only PCB production facility in the United States is the Monsanto plant at Sauget, Illinois which has produced PCB mixtures ranging from 20 to 68 percent chlorine. Polychlorinated terphenyls have been produced at this facility, but production was suspended in 1971.

Soil contamination studies[7] were initiated in February 1976. The sampling locations around the Monsanto facility are identified in Figure 2.5-1. The levels of Aroclor 1242, Aroclor 1260 and decachlorobiphenyl found at these sampling sites are listed in Table 2.5-1. The distribution of all PCBs analyzed appears to be higher near the plant site and generally decreasing with distance from the site. There is some evidence that higher concentrations are present in the soils located to the southeast which corresponds with the predominant wind direction in this area.

A typical chromatogram of a soil sample obtained near the plant is shown in Figure 2.5-2 along with the reference chromatograms of Aroclor 1242 and 1260 run under the same instrument conditions. Using these reference spectra, this sample contains 11 ppm Aroclor 1242, 9.3 ppm Aroclor 1260 and 1.0 ppm decachlorobiphenyl.

All PCB measurements were made using a Varian Model 2760 electron capture gas chromatograph with a 1.8m glass column operated at 200°C. The column had a 3mm Id and was packed with 1.5/1.95% OV-17/WF-1 on chrom W-HP, 80/100 mesh support. The flow rate was 68 ml/min with an inlet pressure of N_2 at 38psig.

Table 2.5-1
PCB Levels in Soils, ppm, Monsanto

Sample Station	Aroclor 1260	Aroclor 1242	Decachlorobiphenyl	Total PCB
1	0.05	<0.01	0.097	0.147
2	0.12	<0.01	0.61	0.73
3	1.4	<0.01	0.90	2.3
4	0.31	0.68	0.38	1.37
5	0.20	<0.01	0.27	0.47
6	1.3	0.82	1.6	3.72
7	2.9	3.0	1.3	7.2
8	9.6	6.1	2.4	18.1
9	0.65	<0.01	0.12	7.7
10	0.28	0.45	0.081	0.811
11	0.10	<0.01	0.049	0.145
12	0.26	<0.01	0.081	0.341
13	9.6	10.0	1.1	20.7
14	0.03	<0.01	0.40	0.43
15	0.39	0.46	0.12	0.97

SOURCE: Unpublished Report, Contract 68-01-2978, USEPA, Office of Toxic Substances; July 1975

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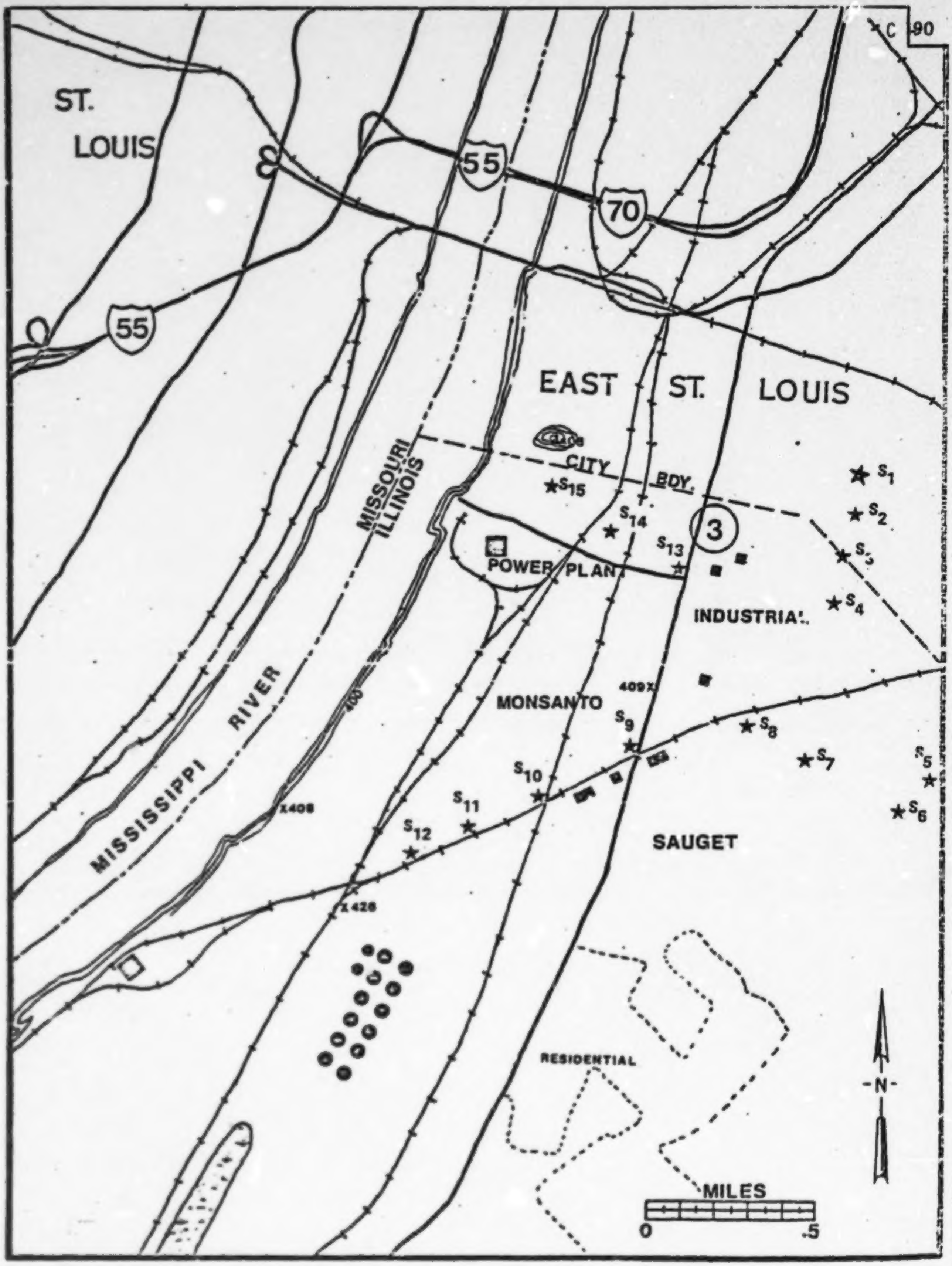


FIGURE 2.5-1 SAMPLING LOCATIONS, MONSANTO

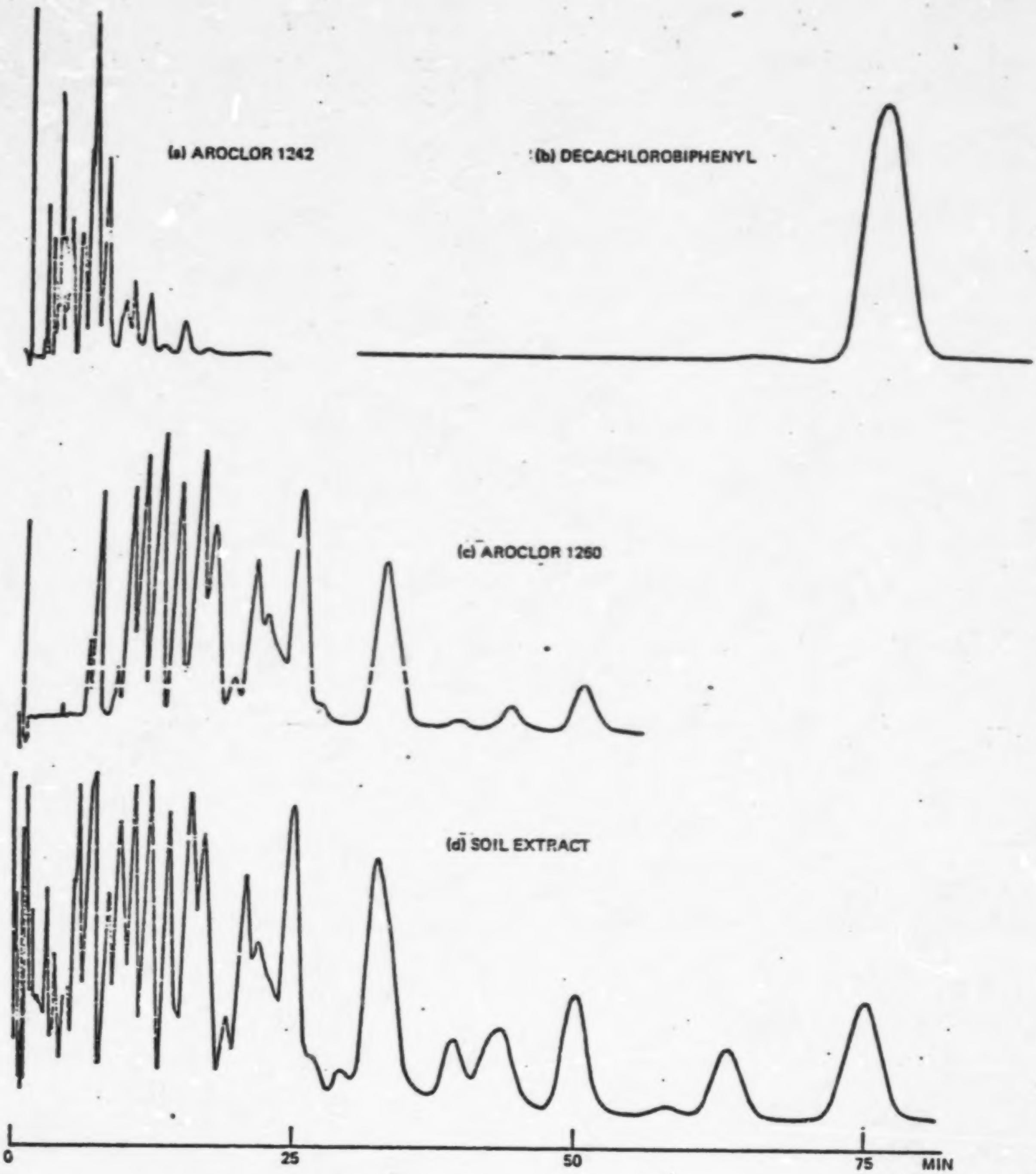


FIGURE 2.5-2 TYPICAL CHROMATOGRAMS OF STANDARD AROCLORS, DECACHLOROBIPHENYL AND A SOIL SAMPLE TAKEN IN THE VICINITY OF MONSANTO CO., SAUGET, ILLINOIS

2.5.1.2 Yates Manufacturing Co., Chicago, Illinois

The Yates Manufacturing Co. is an investment casting wax manufacturer. Prior to 1972, Aroclors 5460, 6090 and 5442, mixtures that contain both PCBs and polychlorinated terphenyls, were used. Subsequent to 1972, this company has been purchasing decachlorobiphenyl from foreign sources.

Soil contamination studies[7] were initiated in February 1976. The sampling locations around the Yates facility used to study soil contamination are identified in Figure 2.5-3. The concentration levels of Aroclor 1242, Aroclor 1260 and decachlorobiphenyl found at these sampling sites are listed in Table 2.5-2.

Table 2.5-2
PCB Levels in Soils, ppm, Yates Manufacturing

Sample Location	Aroclor 1260	Decachlorobiphenyl	Total PCB
1	0.22	<0.001	0.22
2	0.24	0.053	0.293
3	0.81	0.033	0.113
4	0.34	0.19	0.53
5	0.51	0.58	1.09
6	0.40	1.2	1.6
7	0.26	0.51	0.77
8	1.5	0.75	2.25
9	0.57	0.040	0.61
10	0.67	0.020	0.69
11	1.6	3.6	5.2
12	0.29	0.001	0.291
13	0.56	0.034	0.594
14	1.8	<0.001	1.8

SOURCE: Unpublished Report, Contract 68-01-2978, USEPA, Office of Toxic Substances; July 1975

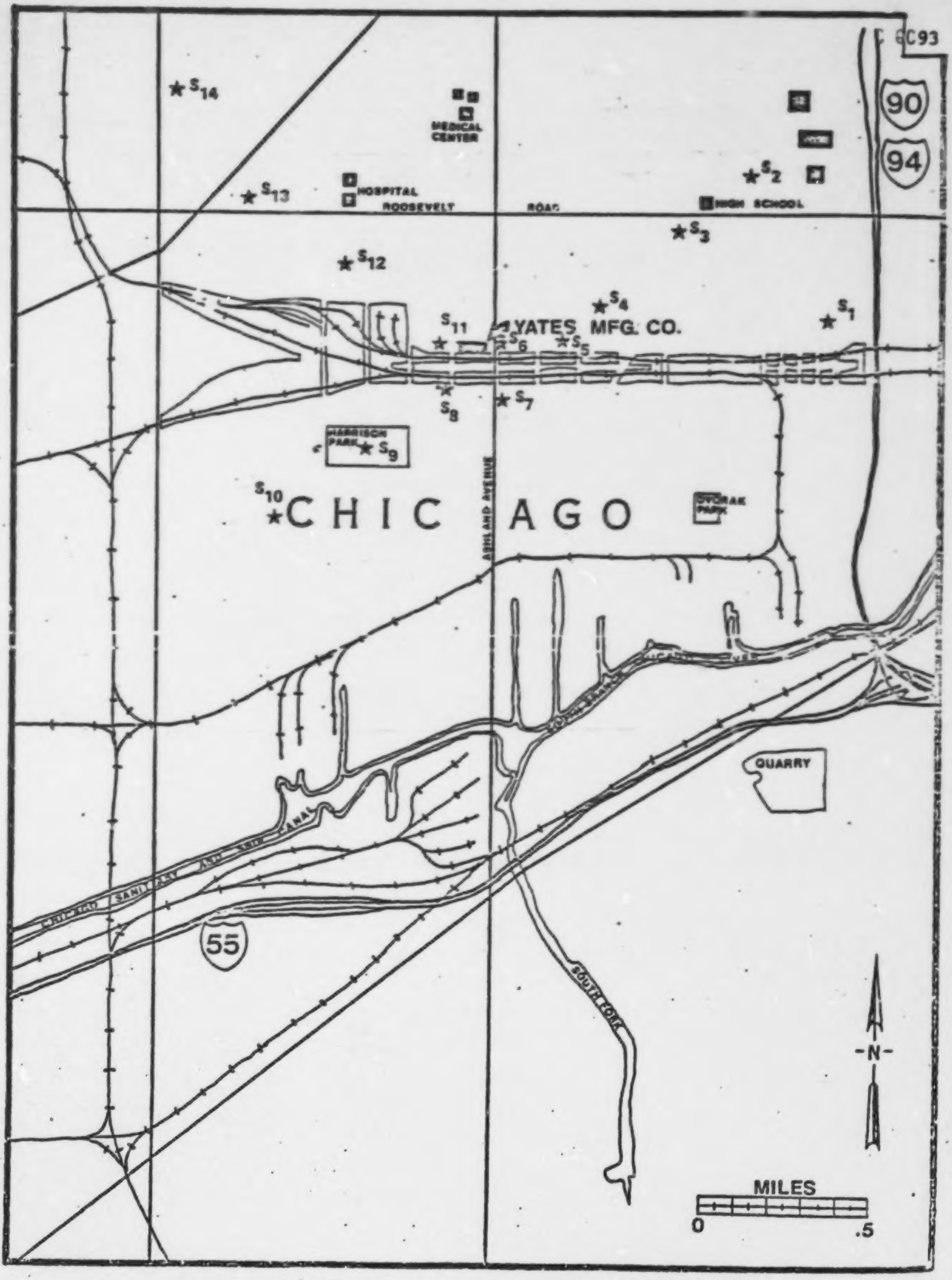


FIGURE 2.5-3 SAMPLING LOCATIONS, YATES MANUFACTURING

2.5.1.3 Valcast Corp., Troy, Michigan

Valcast Corp. is an investment casting facility located in a small industrial park in suburban Detroit, Michigan. PCBs are a constituent of the wax mold compound used to fashion intricate shapes which are to be cast.

Soil contamination studies[7] were initiated in February 1976. The sampling locations around the Valcast facility are identified in Figure 2.5-4. The concentration levels of Aroclor 1260 and 1242 found at these sampling sites are listed in Table 2.5-3.

Figure 2.5-5 is a typical chromatogram of a soil sample extract taken from this location. Aroclor 1260 was present in many of the soil samples taken from the Valcast area, but decachlorobiphenyl was absent. The one observed concentration of 18 ppm of Aroclor 1242 appears to be anomalously high, however, replicate analysis yielded values of 16 ppm and 19 ppm, respectively. Aroclor 1242 was not detected in any other soil samples from this area.

All PCB measurements were made using a Varian Model 2760 electron capture gas chromatograph with a 1.8m glass column operated at 200°C. The column had a 3mm Id and was packed with 1.5/1.95% OV-17/WF-1 on chrom W-HP, 80/100 mesh support. The flow rate was 68 ml/min with an inlet pressure of N_2 at 38psig.

A small drainage ditch that passes adjacent to the north boundary of the Valcast facility was sampled. This ditch serves to remove stormwater runoff in the vicinity and receives discharged cooling water from Valcast and other local small industies. Analysis of the Valcast cooling water at the point of discharge and of water in the drainage ditch failed to detect PCB levels greater than the detection limit of 0.1 ppb Two bottom sediment samples taken from this drainage ditch, however, had concentrations as follows:

Aroclor 1242	2.3 ppm	9.4 ppm
Aroclor 1260	6.7 ppm	8.9 ppm
Decachlorobiphenyl	0.09 ppm	0.11 ppm.

Table 2.5-3

PCB Levels in Soils, ppm, Valcast

Sample Station	Aroclor 1260	Aroclor 1242
1	<0.01	
2	<0.01	18.0
3	0.04	
4	0.06	
5	0.05	
6	0.04	
7	0.14	
8	0.07	
9	<0.01	
10	0.12	
11	<0.01	
12	0.12	
13	0.03	
14	<0.01	
15	0.03	
16	0.02	

SOURCE: Unpublished Report, Contract 68-01-2978, USEPA, Office of Toxic Substances; July 1975

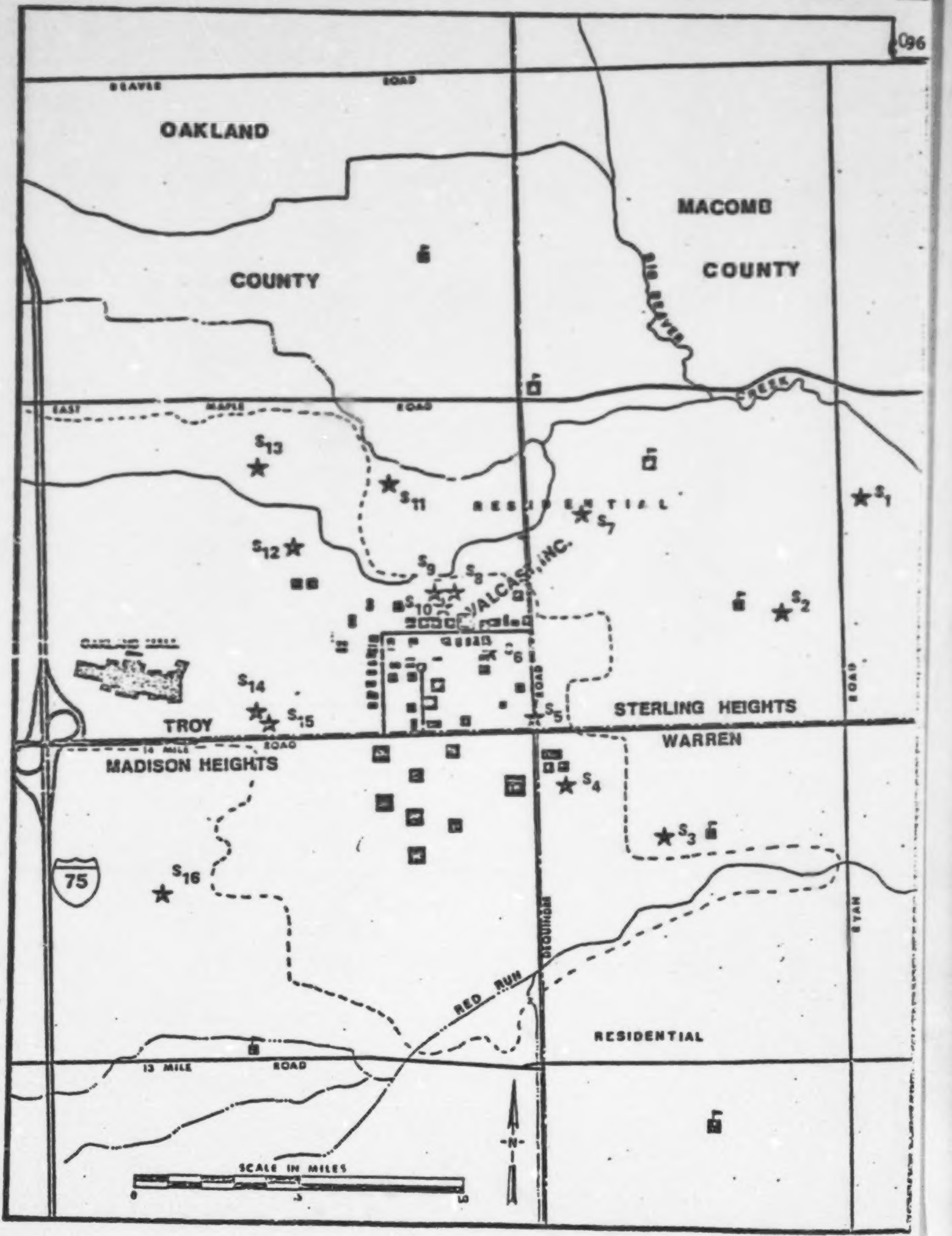


FIGURE 2.5-4 SAMPLING LOCATIONS, VALCAST

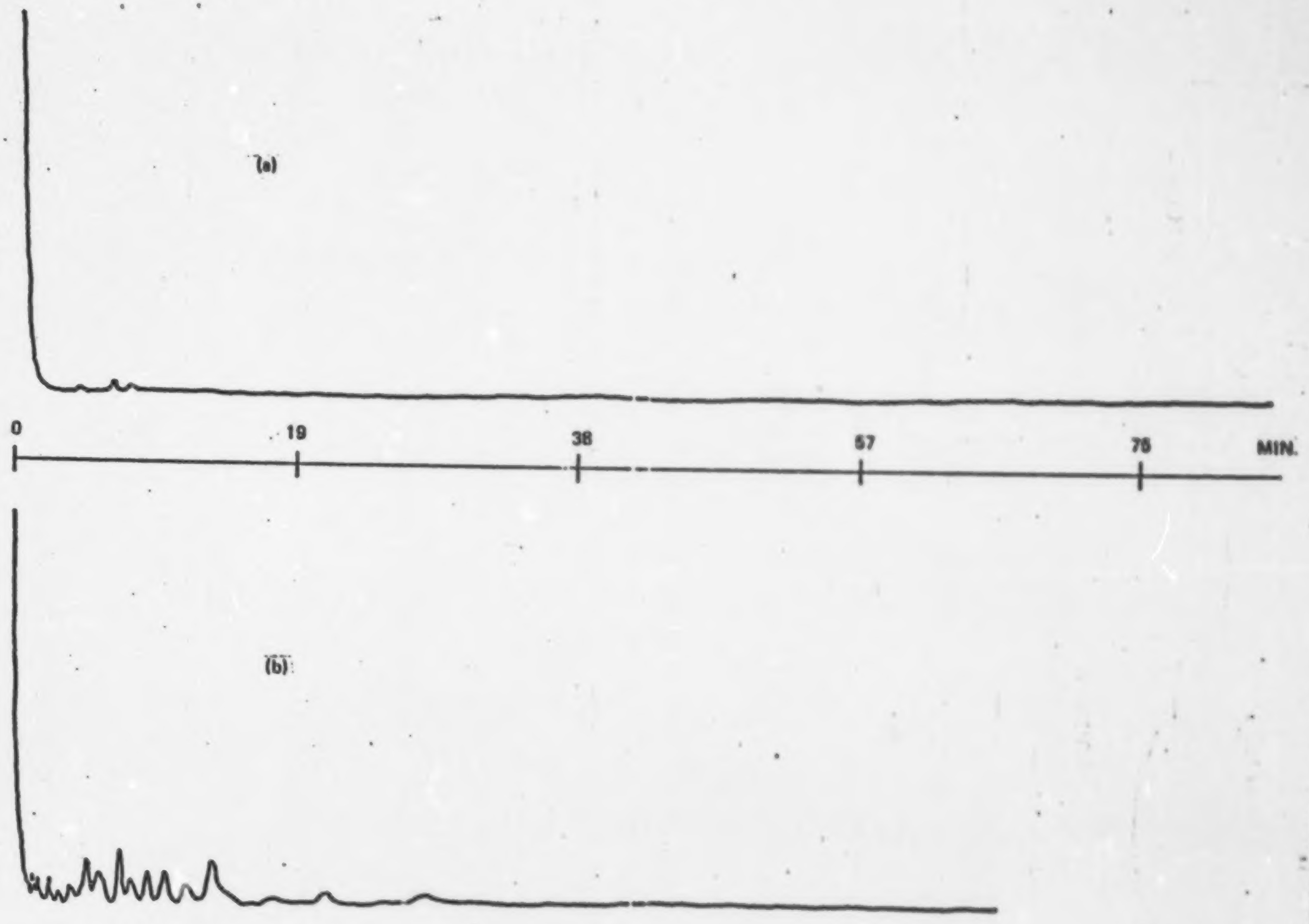


FIGURE 2.5-5 TYPICAL CHROMATOGRAMS OF SOIL SAMPLES TAKEN IN THE VICINITY OF VALCAST CORP., TROY, MICHIGAN – (a) 0.01 ppm, (b) 0.014 ppm AROCLOR 1260

2.5.1.4 General Electric, Hudson Falls - Ft. Edward, N.Y.

The General Electric Plants at Hudson Falls and Ft. Edward use large quantities of PCB's for filling capacitors and transformers. Both plants have chemical waste treatment facilities, but, significant PCB and oil/grease levels are discharged by the waste stream. The NPDES Permit Application lists:

Outfall Location	Daily Average Concentration ppm		Average Daily Loading (lbs.)		Maximum Daily Loading (lbs.)	
	Oil/Grease	PCB	Oil/Grease	PCB	Oil/Grease	PCB
Hudson Falls	13.7	.5	239.8	10.0	250.9	17.6
Hudson Falls	2.1	—	4.9	—	5.25	—
Ft. Edward	8.9	5.0	38.27	20.0	44.5	30.0

In the summer of 1974, EPA Region II initiated a sampling program in the general vicinity of the General Electric plants.[2] The five sampling stations established for this program are shown in Figure 2.5-6. Station 0 is upstream from the plants and serves as the control station; station 1 is located at the junction of the outfall stream from the Ft. Edward facility and the Hudson River, station 2 is located about 0.25 miles downstream from the outfall junction, station 3 is about 0.5 miles downstream from station 1, and station 4 is located about 0.75 miles downstream from station 1.

Samples collected included sediment, water, fish and snails; Arclors identified included 1016, 1254, 1248 and 1242. The levels found at each sampling location are listed in Table 2.5-4.

PCB's identified as Aroclor 1016 were conclusively identified in the water samples at detectable concentrations at all sampling locations except Station 0 (control) and station 4, the furthest downstream.

At all stations the sediments contained higher concentrations of Aroclor 1016 than the water column resulting from adsorption of PCB's on suspended or already settled materials.

The biological samples collected at station 0 contained PCB's characteristic of Aroclor 1254 and Aroclor 1248. Distinctly different from the samples at station 0 were the samples collected in the vicinity of or below the General Electric discharge. Fish data from these areas suggests that Aroclor 1242, Aroclor 1016, or a mixture of these two formulations are present in the Hudson. There are no distinguishing features which can reliably determine whether the mixture is Aroclor 1016 or 1242 at this time.

Table 2.5-4
Environmental PCB Levels from the General Electric, Hudson Falls - Ft. Edward N.Y. Area

Media	Water (ppb)	Sediment (ppm)	Fish (ppm)		
Aroclor	1016	1016	1254	1248	1242
Station					
0	<1.0	6.9	4.0	13.0	
1	2800	6700			45[a]
2	2.2	540			
3	3.0	2980			350[b]
4	<1.0	6.6			78[c] 27[a]

[a] Snails (composite)

[b] Rock Bass

[c] Shiner Minnows

SOURCE: Nadeau R.J., and R.P. Davis, Investigation of Polychlorinated Biphenyls in The Hudson River, USEPA Region II.

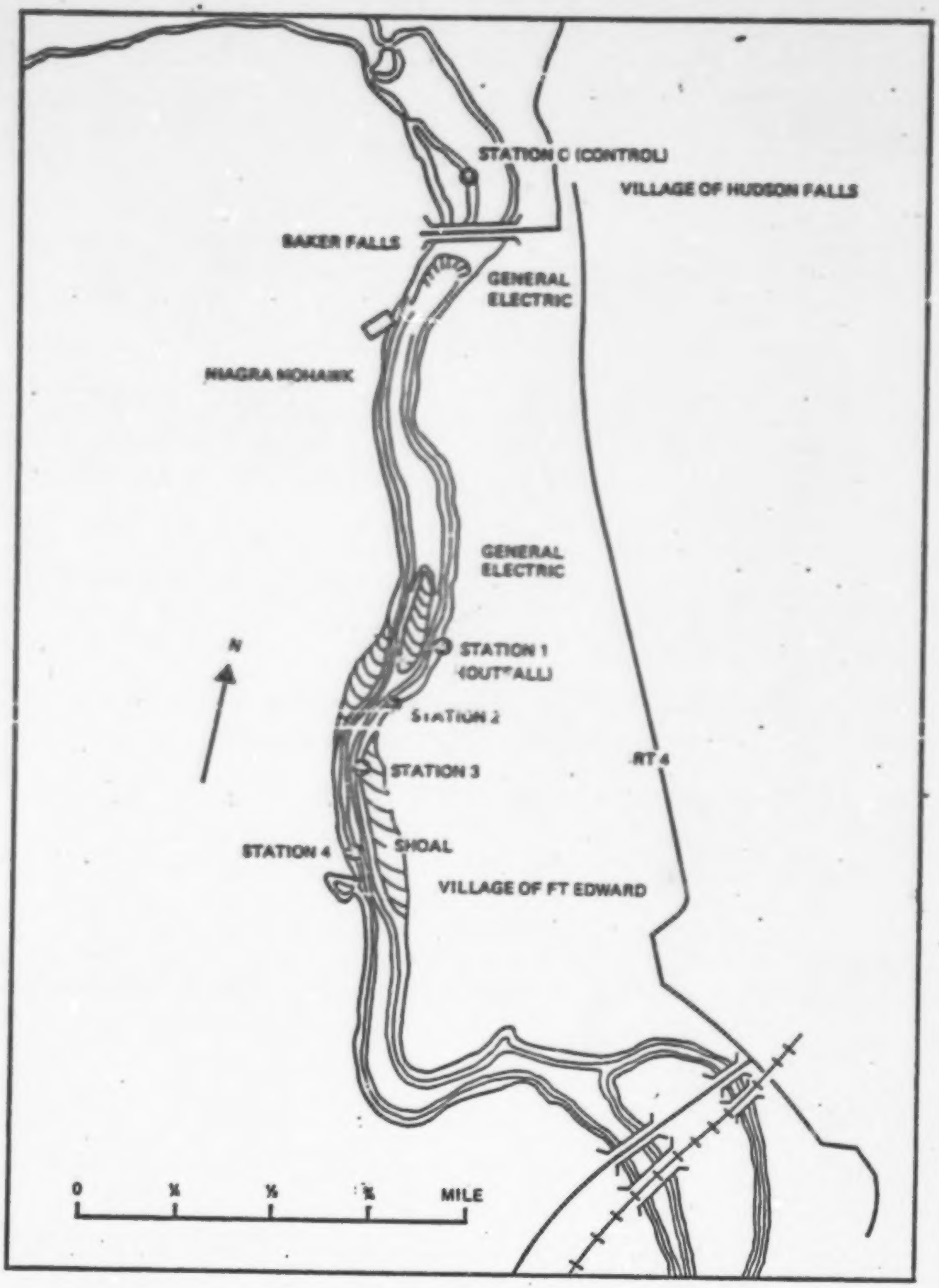


FIGURE 2.5-6 MAP OF HUDSON FALLS-FT. EDWARD, NEW YORK. NOTE LOCATION OF SAMPLING STATIONS RELATIVE TO GENERAL ELECTRIC FACILITIES.

2.5.2 Sewage Treatment Facilities

2.5.2.1 Illinois

An early study[8] of discharges into Lake Michigan was conducted by the state of Illinois in 1970-1972 from locations as shown in Figure 2.5-7. Only two counties in Illinois border on the lake and all sewage treatment plant effluents and tributary stream sources enter the lake from Lake County. In most cases, sewage treatment plant effluents had higher concentrations of PCB's than the tributary streams.

PCB's were not measured in 1970; however, in 1971, 37 sediment samples were collected from tributary streams and ravines in Lake County and at stations offshore from Lake and Cook counties and analyzed for PCB's. The lake samples were collected 40 to 80 yards offshore from seven North Shore Sanitary District sewage treatment plants and at stations approximately one to three miles offshore. Aroclor 1242 in tributary sediments ranged up to 553 ppb in an unnamed channel in Waukegan and Aroclor 1254 ranged from 1.54 to 232.00 ppb in Pettibone Creek in North Chicago. Samples from open water sediments had Aroclor 1242 concentrations ranging up to 106.07 ppb. The highest level was found 40-80 yards offshore from the North Shore Sanitary District sewage treatment plant at North Chicago. Aroclor 1254 concentrations ranged from 2.48 to 46.92 ppb. Selected values are given in Table 2.5-5.

Water samples were collected in both 1971 and 1972 at the tributary streams and sewage treatment plants. Aroclor 1242 and 1254 ranged from 14.00-1810.0 ppt and 192.0-388.0 ppt in 1971 respectively and up to 653.0 ppt for Aroclor 1242 and 61.0 to 841.0 ppt for Aroclor 1254 in 1972 in tributary streams. However, in samples from sewage treatment plants Aroclor 1242 concentrations ranged from 268.0-4020.0 ppt in 1971 and up to 21.0 ppt in 1972. Aroclor 1254 concentrations ranged from 139.0-568.0 ppt in 1971 and from 97.0 to 178.0 ppt in 1972. The highest readings were from the North Shore Sanitary District at Waukegan in 1971. Table 2.5-6 presents the levels identified in water samples from Illinois Streams and Sewage Plants Tributary to Lake Michigan.

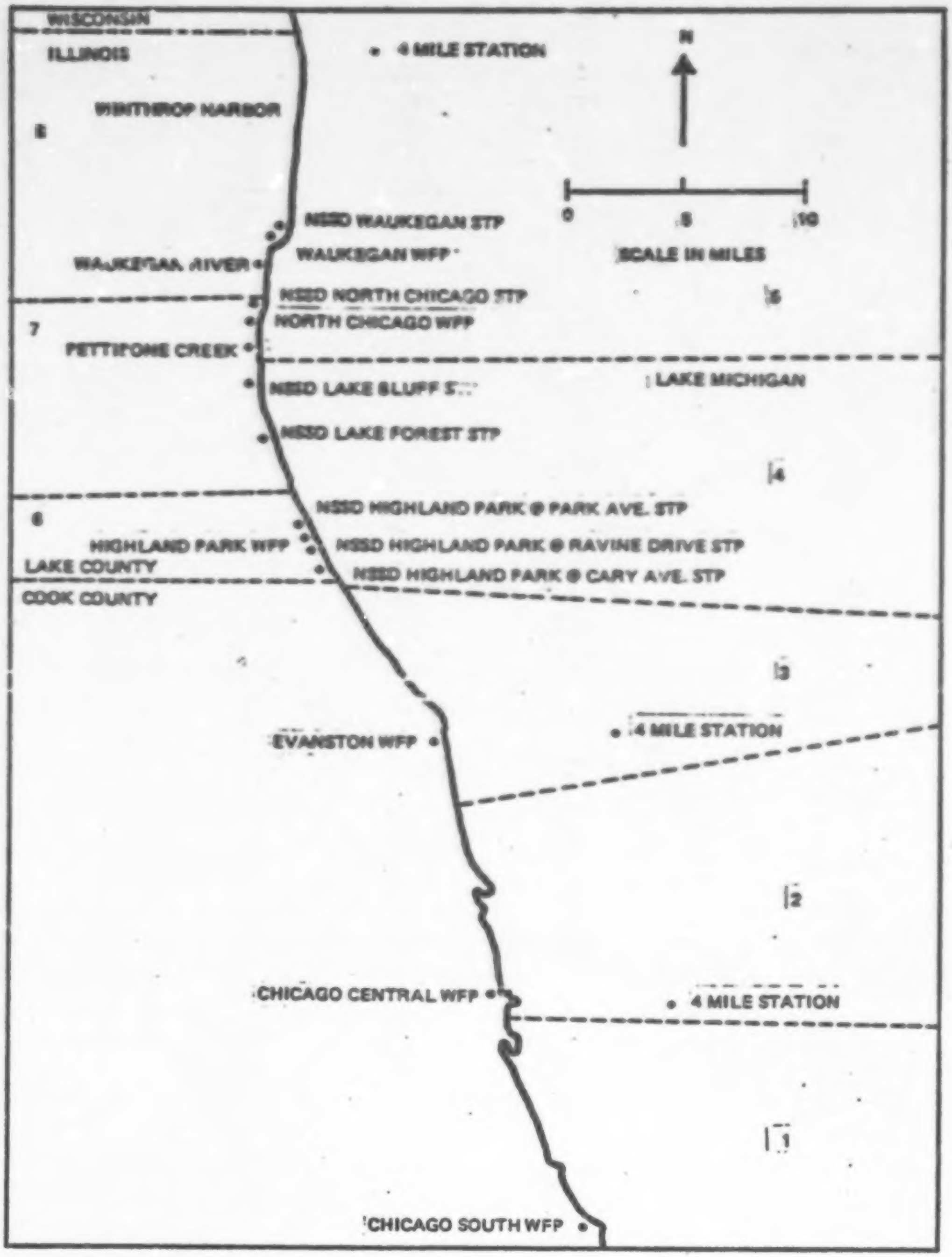


FIGURE 2.5-7 LOCATIONS OF WATER AND SEDIMENT SAMPLING STATIONS

Table 2.5-5
Polychlorinated Biphenyls in Sediments from Lake Michigan and Tributary streams in Illinois 1971 (ppb) dry weight basis

Location		Aroclor 1242	Aroclor 1254
<1 to 3 miles offshores			
Cook County	1	N.D.-18.55	2.48-17.25
	2	17.23-83.35	9.38-46.92
	3	3.58-13.65	3.15-12.42
Lake County	4	7.43-19.25	5.26-17.45
	5	4.98-46.11	8.36-34.52
Highland Park	STP	11.11	12.42
Lake Forest	STP	10.51	7.02
Lake Bluff	STP	44.36	14.45
North Chicago	STP	106.07	26.54
Waukegan	STP	17.32	11.97
10-50 yards upstream from Lake			
Lake County	6	N.D.-4.32	1.54-17.90
	7	1.31-173.40	2.54-232.00
	8	1.77-553.00	2.56-131.00

SOURCE: USEPA, Pesticide Monitoring Programs: Lake Michigan and Tributaries in Illinois, EPA 600/3-74-002

Table 2.5-6
Polychlorinated Biphenyls in Water Samples from Illinois Streams and Sewage Plants Tributary to Lake Michigan 1971 - 1972, (ppt)

Location	Aroclor 1242	Aroclor 1254
1971		
Waukegan River	1810.0	388.0
Pettibone Creek	140.0-187.0	192.0-194.0
Waukegan STP	601.0-4020.0	139.0-568.0
North Chicago STP	268.0-1070.0	153.0-260.0
1972		
Waukegan River	57.0-120.0	61.0-136.0
Pettibone Creek	N.D.-653.0	107.0-841.0
Waukegan STP	N.D.-17.0	97.0-139.0
North Chicago STP	N.D.-21.0	100.0-178.0

SOURCE: USEPA, Pesticide Monitoring Programs: Lake Michigan and Tributaries in Illinois, EPA 600/3-74-002

2.5.2.2 Michigan

Samples collected in 1971 and 1972 from municipal waste water treatment plant effluents throughout Michigan* indicated these plants as a major source of PCB's with an average concentration of 2.55 ppb for 60 effluents sampled. Only seven of the effluents exceeded 1 ppb but one, the Bay City treatment plant on the Saginaw River, had an average effluent concentration of 120 ppb with a high of 340 ppb.

Samples collected from 58 waste water treatment plants in 1973 averaged 0.52 ppb. The Bay City PCB discharge had been greatly reduced as a result of control measures in industries served by the waste water treatment plant.

Sewage Sludge from 57 of the 58 plants tested in 1973 showed Bay City to have the highest concentration of PCB's in the sludge being removed by the treatment process averaging 352 ppm compared to a state wide average of 15.6 ppm.

The range of values for all waste water treatment plants sampled in 1971-1973 are given in Table 2.5-7.

Table 2.5-7
PCB Levels from Waste Water Treatment Plants, Michigan

	Effluent (1971-72) (ppb) Aroclor	Effluent (1973) (ppb) Aroclor		Sludge (1973) (ppm) Aroclor	
City	1254	1242	1254	1242	1254
Adrian	0.41-14.00				
Albion	0.44		0.34		1.5
Ann Arbor	<0.10-0.14		0.25		1.1
Battle Creek	0.16-0.92		<0.10		2.8
Bay City	5.70-340.00	3.20		352.0	
Benton Harbor St. Joseph	0.31-0.99		0.20		13.8
Brighton	0.38				<0.1
Charlotte	0.61		0.34		6.8
Constantine	0.85		0.46		2.1
Detroit	0.88-3.00	2.15	32.1		
Dexter	<0.10		0.23		3.2
E. Lansing	0.35-0.69		0.18		4.6
Escanaba	0.29				5.9
Essexville	0.21-0.28		0.10		3.9
Flint	<0.10-1.30		<0.10		6.3
Flushing	0.52				
Gladstone	0.19	0.44			4.1
Grand Haven	<0.50		<0.10		4.1
Grand Rapids	0.37-0.68	1.05			11.8
Holland	0.42-0.79	<0.10			0.8
Houghton-Hancock	<0.10	0.18			5.5
Iron Mountain-Kingsford	0.55-1.20	0.69			9.5
Ironwood	0.16		0.29		5.2
Jackson	<0.10	<0.10			3.0
Kalamazoo	0.19-1.30	1.12	23.3		
L'Anse	<0.10		0.31		4.4
Lansing	0.13-0.23	0.22			5.3
Manistique	<0.20	0.63			1.5
Marquette	0.35	0.29			2.8
Marshall	<0.10	0.15			3.9
Menominee	0.35	0.57			4.2
Midland	0.13-0.40	<0.10			2.9
Milford	<0.10		2.20		
Monroe	0.33-0.60	<0.10			3.3
Mt. Clemens	1.40-10.00		2.90	175.0	
Mt. Pleasant	<0.10	0.29			6.5
Muskegon	0.28	0.83			12.7
Muskegon Heights	0.37	0.18			11.0
Niles	0.68	0.48			7.8
Norway	0.40	<0.10			<0.1

Table 2.5-7 (cont.)

City	Effluent (1971-72) (ppb) Aroclor	Effluent (1973) (ppb) Aroclor		Sludge (1973) (ppm) Aroclor	
	1254	1242	1254	1242	1254
Ontonagon	<0.10				
Owosso	<0.10	0.12			2.0
Parchment	<0.10				
Pontiac (Auburn Rd)	<0.10-0.61		0.60		
Pontiac (E. Blvd)	0.15-1.30		0.54		
Portage	1.90				
Port Huron	0.28-0.52		1.80		
Saginaw	0.74-3.80		0.77		5.0
St. Ignace	<0.20	0.31			1.5
South Haven	<0.10				
Swartz Creek	<0.10				
Three Rivers	<0.30	<0.10			4.1
Trenton	0.14-1.10	<0.10			<0.1
Warren	0.10-0.16	<0.10			<0.1
Wayne County (Wyandotte)	0.17-0.64	0.40		2.01	
Wyoming	0.44-0.55	0.22			
Ypsilanti	0.21-0.22		0.31		2.0
Ypsilanti Twp #1	<0.10-0.12	<0.10			<0.1
Ypsilanti Twp #2	0.16-0.19	<0.10			<0.1
Cadillac		0.53			<0.1
Howell					15.0
Sault Ste. Marie		0.73			2.4
Traverse City		<0.10			1.8

SOURCE: Monitoring for Polychlorinated Biphenyls in the Aquatic Environment, Michigan Water Resources Commission, May 1973

2.5.2.3 Wisconsin

Samples from sewage treatment facilities in Wisconsin were collected in March 1970. The PCB concentrations identified in the effluents ranged from 0.04 to 0.25 ppb and are presented in Table 2.5-8.[9]

In 1971 11 municipal sewage treatment plants in eleven southeastern Wisconsin cities were sampled as shown in Figure 2.5-8. Table 2.5-9 shows the results of this study and indicates that six of the eleven sewage plants had effluents ranging from 0.1 to 0.5 ppb of Aroclor 1254 while two sites were greater than 1.0 ppb for the same Aroclor. However Portage had 42 ppb of Aroclor 1248 in the effluent with 5.2 ppm in the digester sludge.

It is interesting to note that even though Port Washington is not as highly industrialized as Grafton the effluents from both cities contained approximately the same concentrations of PCB's ranging from 0.12 to 0.23 ppb.

Since the concentrations for Cedarburg were so high a special 24 hour study was conducted. The concentrations in raw sewage began to increase at the beginning of the working day from 0.54 ppb to a maximum of 3.1 ppb at 4:00 p.m. Table 2.5-10 presents the readings taken. The concentration in the final effluent appears to begin increasing from 0.33 ppb at midnight to a maximum of 0.77 ppb at 2 p.m. The concentration of PCB's in the effluent is approximately 30 percent of that in the influent. The concentration of PCB's in the sludges is approximately 1,000 times higher than in the fluid wastes. These data demonstrate that the time of sampling waste effluents is of importance in mass transport estimates.[1]

Table 2.5-8
Concentrations of PCBs in Outfalls into the Milwaukee River on March 26, 1970

Location	Aroclor	PCB Concentration (ppb)
West Bent STP effluent	1254	0.25
Fredonia STP effluent	1254	0.12
Tributary at Fredonia	1260	0.04
Saukville STP effluent	1260	0.13
Chemical plant effluent, Saukville	1242	2.50
Grafton STP effluent	1254	0.04

SOURCE: Veith, G.D., and G.F. Lee, Water Research, 5, 1107-1115, (1971)

Table 2.5-9
PCB Concentrations in the Effluents from 11 Southeastern Wisconsin Sewage Treatment Plants, 1971

City	PCB Concentrations (ppb)	Estimated Mass Transport (in lb/day)
Beaver Dam	0.05	0.0002
Port Washington	0.12-0.22	0.0027
Grafton	0.07-0.23	0.0008-0.0015
Cedarburg	0.28-1.1	0.0027-0.018
Racine	0.60-0.83	0.142
Burlington	0.08-0.14	0.0017
Lake Geneva	2.2-2.8	0.018
Walworth	0.17-0.34	
Beloit	3.89-11.75	0.0038-0.0052
Ft. Atkinson	1.24-2.48	
Portage	32-42	0.0015

SOURCE: Dube, D.J. Polychlorinated Biphenyls in Effluents from Sewage Treatment Plants in Southeastern Wisconsin

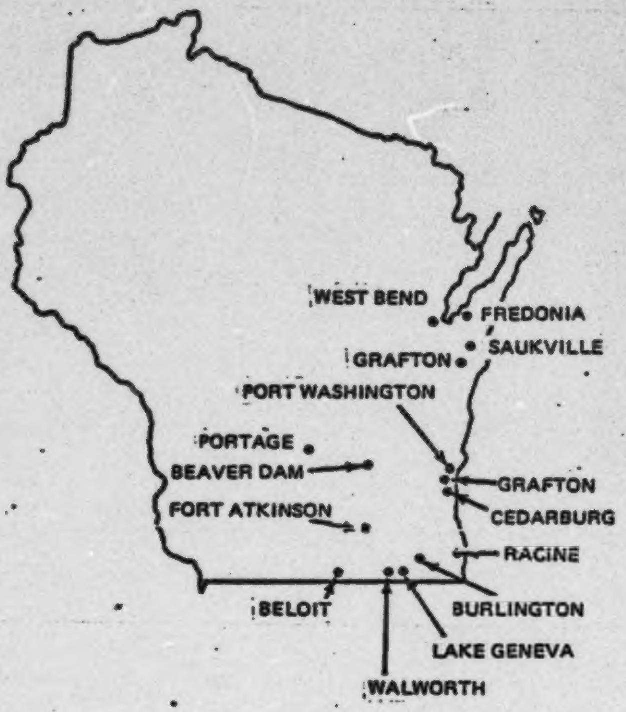


FIGURE 2.5-8 WISCONSIN CITIES WHERE MUNICIPAL SEWAGE TREATMENT PLANTS WERE SAMPLED IN 1971

Table 2.5-10
A 24-hour Survey of PCB's in the Cedarburg Treatment Plant on April 15, 1971

		PCB Concentrations (in ppb)[1]		
Time	Flow (in gal/min)	In the Influent	In the Effluent	In the Primary Trickling Filter
0:00	1480	0.30	0.33	——
1:00	1360	——	——	0.19
2:00	1300	0.20	0.10	——
4:00	1250	0.13	0.35	——
6:00	1200	——	0.16	——
8:00	2000	0.54	0.39	——
10:00	2100	0.40	0.50	——
12:00	1900	1.7	0.23	0.69
14:00	1900	1.5	0.77	——
16:00	1850	3.1	0.70	——
17:00	1900	——	——	0.25
20:00	1950	0.30	0.22	——
21:00	1800	——	——	0.14
22:00	1650	0.36	0.34	——

---

[1] The Chromatograms of the samples most closely resembled the chromatogram of Aroclor 1254.

SOURCE: Dube, D.J. Polychlorinated Biphenyls in Effluents from Sewage Treatment Plants in Southeastern Wisconsin

2.5.2.4 Ohio

Very little information is available from the Ohio River. The values reported are given in Table 2.5-11.

Table 2.5-11
PCB Levels from Sewage Treatment Plants, Ohio, 1971

Collection Site	Aroclor Detected	Concentration (ppb)
Dayton	1254	17
Hamilton	1248	10
Middleton	——	N.D.

SOURCE: Polychlorinated Biphenyls and the Environment, Interdepartmental Task Force on PCB's, May 1972

2.5.2.5 California

In 1970 over a billion gallons of waste water entered the sea each day from urban sewage systems in California. Since two kilograms of PCB output per day are equivalent to one ton per year, outfalls of 9 sewage treatment plants discharging waste to the sea in California were sampled in an effort to identify potential PCB sources. The samples were all taken from sewage outfalls at sites closest to the points of entry into the sea as shown in Figure 2.5-9. The highest output was reported in Los Angeles County which is one of the most industrialized areas in the state. Table 2.5-12 shows the PCB levels identified in the samples collected.[5]

The only other available data were collected in early 1975 on sludge as part of the California Compliance Monitoring Survey for Chlorinated Hydrocarbons.[6] The results obtained are listed in Table 2.5-13.

Only one plant was sampled in both studies, the Hyperion Plant in Los Angeles. The 1970 sludge samples had 78.5 and 98.1 ppb concentrations while the 1975 readings ranged from 1,000-1,400 ppb.

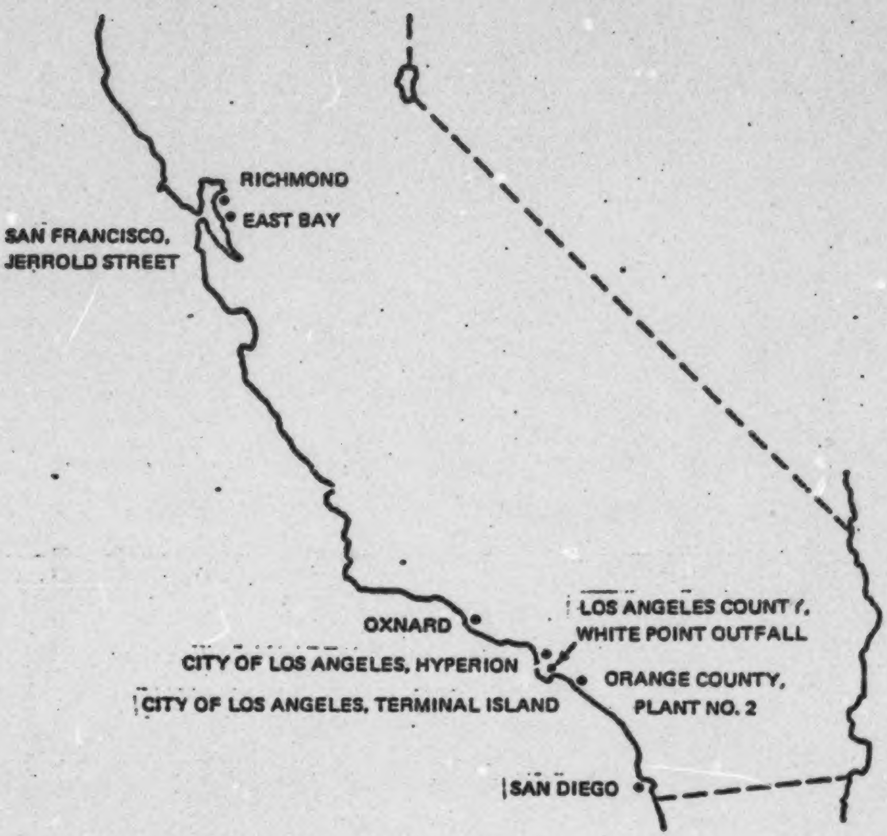

RICHMOND
EAST BAY
SAN FRANCISCO, JERROLD STREET
OXNARD
LOS ANGELES COUNTY, WHITE POINT OUTFALL
CITY OF LOS ANGELES, HYPERION
ORANGE COUNTY, PLANT NO. 2
CITY OF LOS ANGELES, TERMINAL ISLAND
SAN DIEGO

FIGURE 2.5-9 LOCATIONS OF TREATMENT PLANTS SAMPLED, CALIFORNIA

Table 2.5-12
PCB Compounds in Urban Sewage Outfalls in California - 1970

Sampling Station	Description	Flow	PCB Type	PCB ppb	PCB est. kg/day
Richmond	Serves most of Richmond including one of the most heavily industrialized regions of the Bay Area.	21.5	----	N.D	----
EBMUD East Bay Municipal Utility District	Discharges primary treated waste water and digested sludge into San Francisco Bay.	155	1260	3.1-3.8	2.0
San Francisco (Southeast Sewage Treatment Plant)	Serves the industrial area of San Francisco.	31.5	1260	3.8-5.8	0.6
Oxnard	Serves Oxnard.	10.0	----	N.D	----
Hyperion Waste Water (City of Los Angeles) Sludge	Mostly domestic sources.	340 5	1254 1254	0.16-0.37 78.5-92.1	0.4 1.6
White Point	Serves Long Beach, Torrance and other heavily industrialized areas of Los Angeles County.	350	1242, 1254	76	100
Terminal Island	Serves Wilmington and Terminal Island seas. Processes mostly industrial waste.	9.3	1242	5.8-12.8	0.35
Orange County (plant #2)	Serves most of Orange County.	130	1242	0.21-0.64	0.18
San Diego	Serves San Diego and surrounding communities.	80		N.D	

SOURCE: Schmidt, T.T., R.W. Risebrough and F. Gress, Bulletin of Environmental Contamination and Toxicology 6, 235-243, (1971)

Table 2.5-13
PCB Levels in Sludge, California, 1975

Location	Description	Polychlorinated Biphenyls (ppm) Aroclor 1254	 Aroclor 1260	 Aroclor 1242
Orange County Sewage Treatment Plant	Fountain Valley Digester Sludge	——	——	38-40
Hyperion Sewage Treatment Plant	Los Angeles, Digester Sludge	1.0-1.4	——	——
Fort Ord Sewage Treatment Plant	Secondary Digester Sludge	0.2-0.4	——	——
Treasure Isle Sewage Treatment Plant	Sludge	0.4-0.6	——	——
Travis Air Force Base, Sewage Treatment Plant	Digester Sludge	0.6-0.8	——	——
Mather Air Force Base	Sacramento Primary Digester Sludge	0.4-0.6	——	——
McClellan Air Force Base	Secondary Sludge	——	4.5-5.5	——
Beale Air Force Base	Primary Digester	0.3-0.5		

SOURCE: Unpublished Data, USEPA National Field Investigation Center, August 1975

REFERENCES, Section 2.5

1. Dube, D.J. Polychlorinated Biphenyls in Effluents from Sewage Treatment Plants in Southeastern Wisconsin
2. Nadeau, R.J., and R.P. Davis, Investigation of Polychlorinated Biphenyls in the Hudson River, USEPA Region II
3. Polychlorinated Biphenyls and the Environment, Interdepartmental Task Force on PCB's, May 1972
4. Monitoring for Polychlorinated Biphenyls in the Aquatic Environment, Michigan Water Resources Commission, May 1973
5. Schmidt, T.T., R.W. Risebrough and F. Gress, Bulletin of Environmental Contamination and Toxicology 6, 235-243, (1971)
6. Unpublished Data, USEPA National Field Investigation Center, August 1975
7. Unpublished Report, Contract 68-01-2978, USEPA, Office of Toxic Substances; July 1975
8. USEPA, Pesticide Monitoring Programs: Lake Michigan and Tributaries in Illinois, EPA 600/3-74-002
9. Veith, G.D., and G.F. Lee, Water Research, 5, 1107-1115, (1971)

2.6 Data from Localized Monitoring Efforts - Cities

2.6.1 Jacksonville, Florida

Samples of ambient air, water, soils and bottom sediments were collected in the vicinity of Jacksonville, Florida, on September 10, October 6-7 and November 20-21, 1975, for PCB analysis.[1]

Figure 1 illustrates the sampling locations in the Jacksonville area. The outlined area on Figure 2.6-1 circumscribes a municipal sewage sludge disposal site which has been used for the past several years by the City of Jacksonville.

AIR DATA

Air samples were collected at the fire station off Fort Caroline Road using a high-volume sampler equipped with polyurethane foam as the collection medium. Measured PCB concentrations are reported in Table 2.6-1 for the 24-hour period of November 20-21, 1975. The levels measured throughout this period ranged from 4 to 9 ng/m^3, with the lowest levels being observed during the early morning period. Chromatograms indicated the presence of the lower substituted PCB components of Aroclors 1221 and 1016. Quantitation of the PCB levels was performed after perchlorination to decachlorobiphenyl.

WATER, BOTTOM SEDIMENT AND SLUDGE DATA

The analytical data for water, bottom sediment and sludge are summarized in Table 2.6-2 for the sampling points shown on Figure 2.6-1. PCB content of bottom sediments range from 10 to 572 ppb, surface water concentrations ranged from the analytical detection limit of 20 ppt to 285 ppt, expressed as decachlorobiphenyl. The highest sediment concentration (572 ppb) was observed near the cooling water discharge of the Jacksonville Electric Authority Southside Generating Station. This may be related to use of PCB-containing materials at the generating station. Intermediate concentrations were observed in sections of the St. John's River which is surrounded by urban and industrial development. Drainage creeks from the municipal sewage sludge landfill area had bottom sediments of lower concentration. Inspection of gas chromatographic elution patterns indicates the presence of Aroclor 1260 in these sediment samples.

A concentration level of 720 ppb was measured for fresh sludge taken from the Monterey sewage treatment plant. Two-year-old sludge, taken from the landfill area, had a concentration level of 119 ppb.

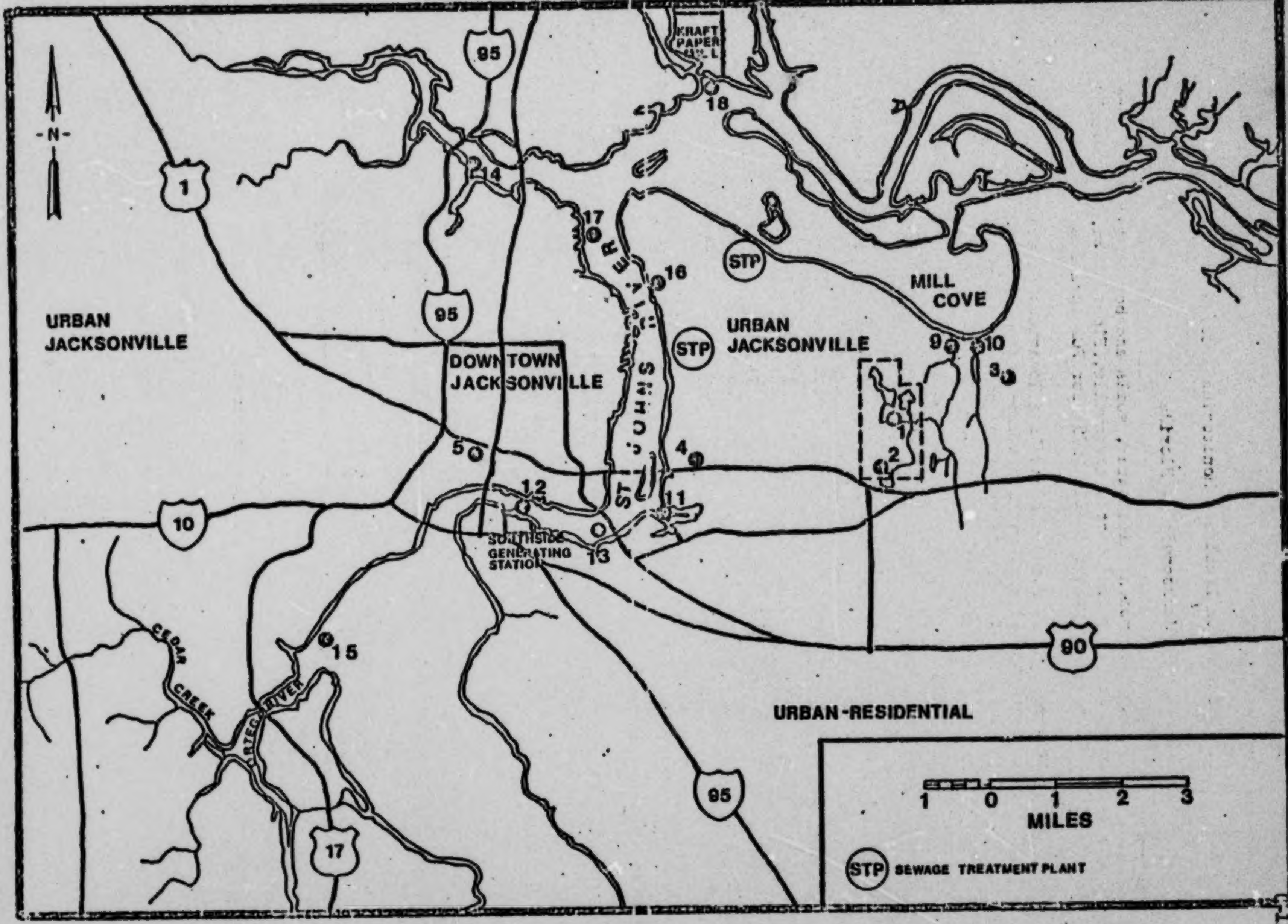


FIGURE 2.6-1 SITE MAP OF JACKSONVILLE, FLORIDA, VICINITY SHOWING SAMPLE LOCATIONS.

Table 2.6-1
PCB in Ambient Air in the Vicinity of Jacksonville, Florida

Data	Period	Concentration (ng/m^3)*
11/20/75	1200-1600	9
11/20/75	1200-1800	8
11/20/75	1800-2400	9
11/20/75	2000-2400	7
11/21/75	2400-0600	4
11/21/75	0600-1200	6

* Reported as decachlorobiphenyl.

Table 2.6-2
PCB Analysis of Environmental Samples in the Jacksonville, Florida, Area.

Sample Site	Sample Type	Site Description	Concentration (ppb)*
1	Sludge	Monterey sewage treatment plant	720
2	Soil	SW section of sludge landfill; about two years old	119
3	Soil	Air collection site	68
9	Sediment	Unnamed Stream into Mill Cove	10
10	Sediment	Ginhouse Creek	21
11	Sediment	Arlington River Mouth	74
11	Water	Arlington River Mouth	0.260
12	Sediment	Generating Station Discharge	572
13	Sediment	St. John's River	142
14	Sediment	Trout River	167
14	Water	Trout River	0.020
16	Water	Jacksonville College Pier	0.285
17	Water	St. Joseph's River	0.103
18	Water	Broward River Mouth	0.020

*Reported as decachlorobiphenyl.

SOURCE: Unpublished Report. Contract 68-01-2978, Office of Toxic Substances, EPA, December 1975.

REFERENCES, Section 2.6

1. Unpublished Report. Contract 68-01-2978, Office of Toxic Substances, EPA, December 1975.

3.0 Behavior of PCB's in the Environment

In general, PCB environmental movement is dependent on stability, solubility and volatility. These properties will control their transport via air, soil, water and/or sediment depending on the partition coefficient in each phase.

General discussions on the thermal, photochemical and chemical properties of PCB's have recently been published by Hutzinger, Safe and Zitko[5]. Various transport mechanisms have received considerable attention[3,4]. Current air data indicates that the predominant movement of PCB's in the atmosphere is not attached to particulates as is the case with DDT, but rather as the free unbound molecular species.

Some of the complexities of the behavior of PCB's in the environment include the following characteristics which are discussed in subsequent sections: (a) the complexity of the composition of different Aroclor products, (b) the difficulty in definition of water solubility and adsorption properties due to the complexity of the mixtures, and (c) the variability of the volatilization process depending on the starting mixture and point of release. By coupling these areas, the focus will be on those PCB properties that influence environmental sample collection, control their distribution in environmental samples and present potential difficulties in environmental sampling.

3.1 Composition of Aroclor Products

PCB's have been manufactured in the United States by the Monsanto Industrial Chemicals Company, and marketed under the trademark Aroclor with a numerical designation[8]. Table 3-1 provides an overview of the major Aroclors produced with their associated chlorine content. These Aroclor products are mixtures of different isomers. A typical analysis of the currently produced products and the associated chlorobiphenyl composition is shown in Table 3-2. In addition, the number of potential isomers making up the chlorobiphenyl component are also indicated. From this chemical analysis, Aroclor 1221 can be considered primarily as a monochlorobiphenyl and is the only Aroclor with a significant biphenyl concentration; Aroclor 1016 and 1242 are primarily trichlorobiphenyls, with the 1016 having a lower penta and hexa content than the 1242 product; Aroclor 1254 is primarily a pentachlorobiphenyl product. The composition of the different isomers could be quite different for each product.

3.2 Water Solubility of PCB's

The water solubility of various Aroclor products and some chlorobiphenyl isomers has been measured by different investigators. A limited set of reported values are shown in Table 3-3. The solubility for all three monochlorobiphenyl isomers is listed and range from 1190-5900 ppb. The solubilities for all of the higher chlorobiphenyl isomers have not been measured and only representative compounds are listed to show the range of values possible and the component solubilities for Aroclor 1221; for the dichlorobiphenyls, the 2,4-, 2,2'-, 2,4'-, 4,4'-dichloro isomer is listed out of a possible 12 isomers and range in solubility from 80-1880 ppb; for the trichlorobiphenyls, only the 2,2',5-trichloro is listed out of a possible 24 isomers; for the tetrachlorobiphenyls only the 2,2',5,5'-tetrachloro is listed out of a possible 42 isomers. These values indicate that PCB's have low solubilities and the solubility decreases with the number of chlorine atoms present in the isomer, i.e., the lower isomers containing chlorine show the highest solubility. Factors that contribute to the variability of the results include adsorption to particulate matter or wall surfaces and the slow equilibration process.

Studies by Haque et. al.,[3] show that equilibration of Aroclor 1254 in water required approximately two months to reach complete equilibrium, with the major portion being achieved in a week. These studies also included various types of adsorbent surfaces ranging from sand to highly organic soil samples. The adsorption increased strongly with the organic content of the adsorbent studied. Starting with an original concentration of Aroclor 1254 at 56 ppb, the addition of 100 grams of sand or silica gel had very little effect on the final PCB concentration. However, by adding a high organic content soil, the final PCB concentration was reduced by approximately 85%.

Studies by Eichelberger[1] on the behavior of PCB's in river water over a 16 week period indicated that the levels of recovery of 10 ppb spiked Aroclor 1242 samples were 87, 76, 73, 60, 53 and 43 percent for zero time, 1, 2, 4, 8 and 16 weeks respectively. Losses were attributed to irreversible adsorption on either the walls of the sample container or the silt contained in the sample.

3.3 Interactions of PCB's with Soils

The behavior of PCB's in soil is a complex process dependent on a number of factors which include surface properties and composition. Soil composition can be identified by characterizing the percentage of sand, silt, clay and organic carbon. Depending on the surface properties, soils can act as an effective barrier to PCB migration in landfill sites and as an effective sink to PCB's deposited via aerial fallout. Once the PCB's are adsorbed, factors that would influence PCB movement are leaching when water is added to the soil and re-evaporation under normal atmospheric conditions.

In a series of experiments directed at understanding the leaching properties for various soils that could be encountered at different landfill sites, different types of soils were studied[10]. These experiments percolated water through a column packed with soil coated with Aroclor 1016 and monitored the effluent water. Breakthrough of the PCB's was found to be related to the clay content of the soil. Soils with the higher clay content retained the PCB's. The isomer distribution in the effluent reflected the different water solubility and adsorption properties characteristic of the Aroclor 1016 isomers. The less chlorinated isomers were more readily leached. Results from these experiments are shown in Figure 3-1, where an Aroclor 1016 and 1221 standard solution is compared with the leached mixture. A .32mm id x 50m glass capillary column coated with OV-101 was used for these separations. The flow rate was 2 ml/min helium and the column temperature was programmed from 150° to 230°C at 2°C/min. The resulting isomer distribution is significantly changed from the starting Aroclor 1016 and has a closer resemblance to the isomer pattern of Aroclor 1221. In the worst case, less than 0.05% of the total Aroclor 1016 available was leached under conditions equivalent to forty years of annual rainfall.

Adsorption and evaporation studies[3] with Aroclor 1254 on different soils show that due to the few adsorption sites in sand, sand surfaces adsorb relatively small amounts when compared to the other types of soils. Consequently the vaporization loss from a sand surface will be significantly higher than soil surfaces where it is more tightly bound. The less chlorinated isomers show a greater loss than those isomers of high chlorine content.

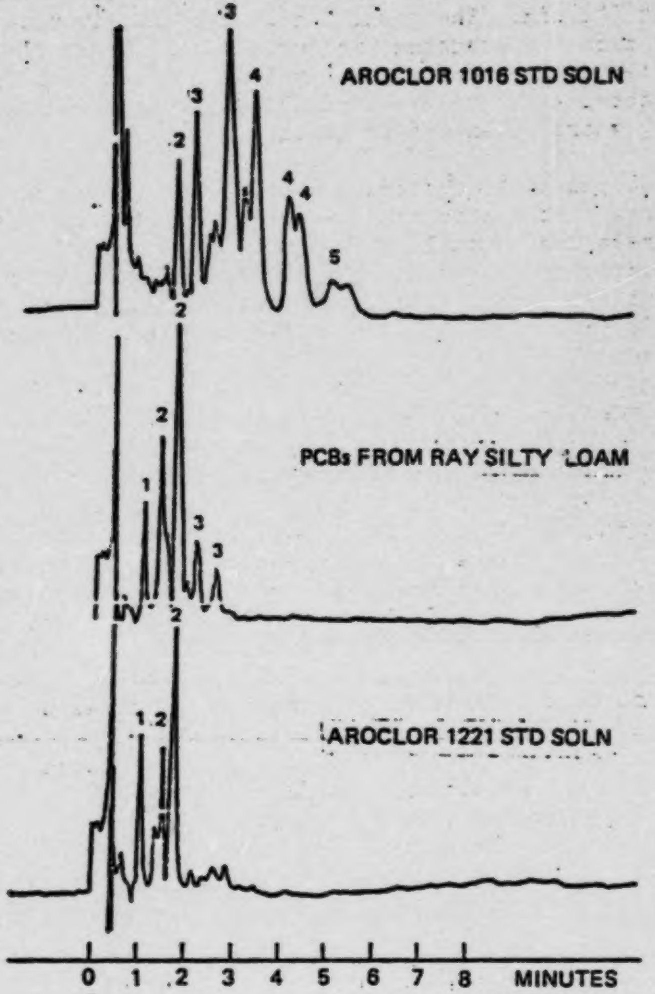
3.4 Evaporation of PCB's from Water

The transfer of PCB's from water to the air environment may be significantly faster than expected when considering that these compounds have a high molecular weight, low solubilities and low vapor pressures[6,7]. On examination, these compounds exhibit very high activity coefficients in aqueous solution resulting in high equilibrium vapor partial pressures and consequently high evaporation rates. The rates are relatively insensitive to temperature. For different Aroclor products, the evaporation process is complex depending on the chlorobiphenyl isomers present, leading to different isomer concentrations in each phase with time. The calculated half-lives for Aroclor 1242 and 1254 for a water depth of one meter are 12.1 and 10.3 hours respectively. Using the half-life values, if a monitoring station is located one hour downstream from a source of Aroclor 1242, by the time the water reaches the station, approximately one twentieth of the initial levels may have been evaporated. Where the water body is turbulent, the evaporation rate will be increased significantly.

3.5 Environmental Sampling Guidelines

Based on these limited studies, the following guidelines and precautions are suggested:

(1) laboratory measurements of field samples should be made as soon as possible;
(2) concentration levels of field samples measured in the laboratory will be lower than actual concentrations;
(3) concentration levels in water may be much lower in some areas depending on the type of suspended particulate matter;
(4) samples collected at plant outfalls will be non-equilibrium samples;



SOURCE: MIEURE, J.P., O. HICKS, R.G. KALEY AND V.W. SAEGER, "CHARACTERIZATION OF POLYCHLORINATED BIPHENYLS," NATIONAL CONFERENCE ON POLYCHLORINATED BIPHENYLS, CHICAGO, ILL., NOVEMBER 19-21, 1975

FIGURE 3-1

(5) sampling methods should be employed which reduce the problem of irreversible adsorption on container walls;
(6) soil samples will have an enriched high chlorobiphenyl isomer concentration;
(7) leached water and air samples will have an enriched lower chlorobiphenyl isomer concentration;
(8) true identification of the specific Aroclor released into the environment becomes more difficult with time.

Table 3-1
AROCLOR PRODUCTS
Produced by Monsanto

Currently in Production	Discontinued	Percent Chlorine
1221		21
	1232	32
1016		41
1242		42
	1248	48
1254		54
	1260	60
	1262	62
	1268	68

Table 3-2
COMPOSITION OF SOME AROCLORS

Chlorobiphenyl Composition	Number of Isomers	Chlorobiphenyl Percent Distribution of some Aroclors			
		1221	1016	1242	1254
$C_{12}H_{10}$		11	<0.1	<0.1	<0.1
$C_{12}H_9Cl$	3	51	1	1	<0.1
$C_{12}H_8Cl_2$	12	32	20	16	<0.5
$C_{12}H_7Cl_3$	24	4	57	49	1
$C_{12}H_6Cl_4$	42	2	21	25	21
$C_{12}H_5Cl_5$	46	<0.5	1	8	48
$C_{12}H_4Cl_6$	42	ND	<0.1	1	23
$C_{12}H_3Cl_7$	24	ND	ND	<0.1	.6
$C_{12}H_2Cl_8$	12	ND	ND	ND	ND

ND = None detected, <0.1%

Table 3-3
SOLUBILITY OF AROCLORS AND CHLOROBIPHENYLS IN WATER

Aroclor Products	Solubility (ppb)	Media	References
1016	225-250	distilled water	10
1221	200	distilled water	8
1242	200	distilled water	9
1254	300-3000	fresh water	12
1254	300-1500	salt water	12
1254	50	distilled water	9
1254	56	distilled water	3
1254	40	distilled water	8
Chlorobiphenyl Compounds			
2-chlorobiphenyl*	5900		5
3-chlorobiphenyl	3500		5
4-chlorobiphenyl*	1190		5
2,4-dichlorobiphenyl	1400		5
2,4-dichlorobiphenyl	637		2
2,2'-dichlorobiphenyl*	1500		5
2,4'-dichlorobiphenyl*	1880		5
4,4'-dichlorobiphenyl*	80		5
2,2',5-trichlorobiphenyl	248		2
2,2',5,5'-tetrachlorobiphenyl	46		11
2,2',5,5'-tetrachlorobiphenyl	26		2

*These are the major chlorobiphenyl compounds in Aroclor 1221; the other major constituent is biphenyl which has a solubility in water of 4600 ppb.

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4 Occurrence of PCB's in Food

Some of the earliest findings of PCB's in foods occurred during the fall of 1969 in coho salmon and milk from West Virignia which was traced back to the misuse of a transformer fluid containing PCB's for defoliant spraying adjacent to dairy pasturage. In 1971 PCB's were found in poultry from North Carolina. Contaminated fish meal was implicated as the causative agent. The contamination was traced back to a leak in heat exchange equipment using PCB's. The fish meal samples examined contained from 14 to 30 ppm of PCB's. Followup sampling of eggs showed that 71 of 224 eggs contained residues in excess of 0.5 ppm ranging from 0.6 to 4.2 ppm.[3]

The Food and Drug Administration conducts a comprehensive food surveillance program yearly to determine pesticide residues, PCB's, heavy metals and other contaminants in the dietary intake of consumers in the United States and to target emerging problems and trends. The FDA has analyzed all raw agricultural commodities sampled under the pesticide surveillance program for PCB's since 1969. PCB's have been encountered most frequently in fish, both freshwater (catfish, chub, and smelt) and saltwater (porgies, sea trout, bonita and sardines), with trace levels in shellfish. Table 4-1 presents the results of this program from July 1970 to September 1971.[3]

As a result of these findings FDA proposed certain regulations for PCB concentrations in foods in 1972. These temporary tolerances are:

Commodity	PCB Concentration (ppm)
Milk (fat basis)	2.5
Dairy Products (fat basis)	2.5
Poultry (fat basis)	5.0
Eggs	0.5
Finished Animal Feed	0.2
Animal Feed Components	2.0
Fish (edible portion)	5.0
Infant and Junior Foods	0.2

Of these commodities, fish is the only food primarily contaminated by the environment (waterways). The other commodities were contaminated by industrial and agricultural uses.

In FY 73 and 74 Comprehensive Fish Surveys were carried out. While the data are valuable in showing which species and which areas are apt to be of concern, the diversity of the fish sources and reasons for collecting them make it difficult to determine if there have been any trends. In the FY 73 program no PCB's were detected in 70% of the samples; 3% contained over 1 ppm while only 0.5% had over 5 ppm of PCB's. Those over 1 ppm were generally fresh water fish or those apt to be near the shore. Carp were the only fish over 5 ppm with a high of 20.5 ppm. The FY 74 survey, which was not carried to completion, showed no PCB's in over 80% of the samples analyzed and no samples contained more than 2 ppm.[2]

The total diet studies for FY 70 and FY 71 showed composite samples containing PCB residues up to 0.36 ppm. The positive readings were found in meat, fish, poultry, dairy and the grain and cereal composites. The 0.36 ppm value was found to be caused by migration of PCB's from the grayboard container and dividers to packaged shredded wheat.[3]

The FY 73 study included thirty market basket samples from representative areas of the United States consisting of the total 14-day diet of a 15-20 year old male in the region of collection including about 117 individual food items.[1]

These 117 food items are separated after any necessary preparation into twelve food group composites for analysis:

I	Dairy Products
II	Meat, Fish and Poultry
III	Grain and Cereal Products
IV	Potatoes
V	Leafy Vegetables
VI	Legume Vegetables
VII	Root Vegetables
VIII	Garden Fruits
IX	Fruits
X	Oils, Fats and Shortening
XI	Sugar and Adjuncts
XII	Beverages (including drinking water)

Most of the PCB levels identified in 1973 were trace amounts resulting in a daily intake of only 1 µg/day. The most frequent occurrences were in the meat-fish-poultry and grain-cereal products groups with 33% and 17% positive analyses. It has been suggested that environmental contamination may be the source in the first group and lingering recycled paper contamination in the second group.

Of 30 composites examined for each commodity group PCB residues were only determined in five of the groups as follows:

Group	Frequency
Dairy Products	3
Meat, Fish and Poultry	10
Grain and Cereal Products	5
Potatoes	1
Oils, Fats and Shortening	1

The range for all 20 positives was trace to 0.073 ppm. In fact 19 of the 20 readings were only trace amounts with the 0.073 ppm composite appearing in the Grain and Cereal Products group.[1]

Data for 1974 show that there were positive readings in only two food groups: sugar and adjuncts; and meat, fish and poultry. While only 3% of the samples in the first group were positive, 43% of those in the meat, fish and poultry group were positive. The fish components of these samples were usually the source of the contamination. Preliminary information indicated that the range of levels measured was trace to 0.05 ppm. Data for the first half of 1975 have found 40% of the meat, fish and poultry group to show positive readings for PCB's with no positives in other food group.

Using the preceding information the FDA has estimated the average daily intake from all twelve food group composites and the average daily intake from the meat fish and poultry food class as presented in Table 4-2. For those levels which were reported as trace the assumption was made to average them at 1/2 the quantitative lower level of detection; i.e., 0.025 ppm. It can be seen that there has been a decrease in the estimated total intake. This intake should level out and continue at the 1975 level as long as fish remain almost the sole source of PCB's and the entry of PCB's into waterways is not decreased.[2]

Table 4-1
PCB Residues in Selected Food Commodities
July 1970 - September 1971

	Number of samples examined	Number of samples positive	Percent positive	Low	PCB levels, (ppm) high	Average (ppm)
Fish	670	363	54	T	35.29	1.87
Cheese	1344	91	6	T	1.0	.25
Milk	941	69	7	T	27.8	2.27
Shell eggs	550	161	29	T	3.74	.55
Fish by-products	—	13	—	T	5.0	1.17
Total (excluding fish by-product)	3505	684	19			1.14

SOURCE: Kolbye, A.D., Jr., Environmental Health Perspectives, 85-88, April 1972.

Table 4-2
Estimates of Daily PCB Intakes
Total Diet Study

	Average Daily Intake of PCB's	
Fiscal Year	Total Diet (μg/day)	Meat-Fish-Poultry Food Class (μg/day)
1971	15.0	9.5
1972	12.6	9.1
1973	13.1	8.7
1974	8.8	8.8
1975 (1st half)	8.7	8.7

SOURCE: Jelinek, D.F. and P.E. Corneliussen, National Conference on Polychlorinated Biphenyls, November 1975.

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5.0 Exposure and Biological Accumulation of PCB's in Man

5.1 National Monitoring Programs

The National Human Monitoring Program for Pesticides is conducted by the Office of Pesticide Programs, EPA. Small samples of adipose tissue from postmortem examinations and from specimens submitted for pathological examination during therapeutic surgery are collected and analyzed for the presence of chlorinated hydrocarbon insecticides. Analysis for PCB's was begun in 1968. Summaries of the data from 1971 are reported in Table 5.1-1 and from 1972 to 1974 in Table 5.1-2.

Note that although classes are different in the two sets of results, the proportion of samples showing traces of PCB's is increasing: 50.7%, 1971; 73.99%, 1972; 75.49%, 1973; 90.93%, 1974. The increase between 1973 and 1974 is dramatic. However, the percentage of tissues which contained quantifiable (greater than 1.0 ppm) of PCBs appears to be remaining relatively constant (31.1%, 58.53%, 35.08%, 40.30%, 1971-74).

5.2 Localized Studies

There have been several localized studies showing the presence of PCB's in both human plasma and milk. A 1968 study in Charleston County, South Carolina[3], reported that of 612 plasma samples collected from healthy volunteers, 45% showed some PCB residue. Levels ranged to a maximum of 29 ppb with a mean plasma residue of 2.12 ppb. This study also tested for the significance of several factors on PCB levels, concluding only that residues were more frequent and higher in whites and urban residents. A follow-up study conducted in 1972[1] in the Charleston area sampled plasma and scalp hair specimens from 37 refuse burners and 54 controls. PCB residues were not detected in hair samples, but 81% of the refuse burners (compared with only 11% of the controls) had detectable PCB residues in plasma. Median levels for those with detectable amounts in plasma were 2.6 ppb for the refuse workers and 3.7 ppb for the controls, with maximums of 14.1 ppb and 20.2 respectively.

A 1971-72 study[6] of 40 human milk samples in Colorado discovered PCB residues in 20% of the samples in ranges from 40 to 100 ppb. A 1971 study of 47 lactating women in Texas[2] reported an absence of PCB residues both in milk from all subjects and in serum from 28 subjects. A minor study of blood samples from nine cachectic patients and 15 healthy nonpatients from Missouri and surrounding states[4] reported detectable PCB residues in all patients (mean 48 ppb, range 10-100 ppb) and none detectable in the nonpatients. No conclusions were drawn.

Table 5.1-1
PCB Concentrations in Human Adipose Tissue, 1971

	Absent		Trace to Below 1 ppm		1-2 ppm		2 ppm	
Sample Size	Number	%	Number	%	Number	%	Number	%
637	314	49.3	125	19.6	165	25.9	33	5.2

SOURCE: Yobs, Anne R., Environmental Health Perspectives, 1, 79-81 April 1972.

Table 5.1-2
PCB Concentrations in Human Adipose Tissue, 1972-1974

		Absent		Below 1 ppm		1-3 ppm		73 ppm	
	Sample Size	Number	%	Number	%	Number	%	Number	%
1972	4102	1067	26.01	634	15.46	2079	50.68	322	7.85
1973	1277	313	24.51	513	40.17	378	29.60	70	5.48
1974	1047	95	9.07	530	50.62	371	35.43	51	4.87

SOURCE: Kutz, F.W., Project Officer, National Human Monitoring Program for Pesticides, Office of Pesticides Programs, U.S. EPA, Washington, DC.

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6.0 Environmental Trends

There are few data bases or environmental studies which provide an adequate basis for sound analytic determinations of national trends in environmental levels of PCB's. Such a data base would be a result of a system of studies involving consistent sampling across media, i.e., statistical samples taken at the same locations at representative time periods, analyzed according to standard protocols, taken over a period of time long enough to establish a trend. Some studies do show consistent sampling within specific media, e.g., the Great Lakes Environmental Contaminant Survey, but the literature is full of snapshot studies with neither predecessor nor successor, and baseline studies which have not been followed by any further work. Thus, whether due to funding, modification of priorities or personnel changes, long-term consistency and coordination have not been achieved. As a result, the conclusions drawn in this section represent qualitative judgements drawn from the large mass of generally uncoordinated PCB studies analyzed.

Even recognizing all the faults of the available data base, the sheer mass of data supports the conclusion that there is widespread contamination of the environment by PCB's. There are regional variations, but effects are consistent across all media (e.g., water, sediment, fish, birds), generally showing greater concentrations of PCB's in highly developed areas and in areas of industrial activity. Over the past five years of increasing recognition of and attention to the problems of PCB's, the situation has shown no improvement nationally. Conscious efforts have, however, resulted in substantial improvement in some localized instances.

PCB contamination of the nation's waterways was termed widespread by the USGS over the period 1971-1972; more recent data from the USGS up to 1975 has shown no reduction in either levels or in geographical dispersion. Although sampling stations in the USGS data base are not expected to be representative of the U.S. and the data suffers from multiple observations at some stations and spot readings at others, over the period 1971-1975 states which had reported PCB's in sediment continued to do so while six of the 23 states which had shown no contaminated samples in 1973 did report some contamination in 1974. Sediment concentrations are imperfect indicators of current contamination levels and whole water measurements would be better. However, the currently practiced analytical protocol for water measurements, which limits detectability to 0.1 ppb, leads to a preponderance of zero readings in whole water which mask the very real problem of PCB contamination in the nation's waters. In almost all cases in which samples of both whole water and bottom sediment were taken simultaneously and the sediment had a non-zero PCB level, concentration in the water was reported as zero ppb. This occurred even when the concentration in bottom sediment was as high as 4000 parts per billion! Were water measurements to be taken at the parts per trillion level as some recent studies have done, whole water readings would certainly show PCB contamination, to an extent similar to that shown by sediment readings.

The results of the U.S. Fish and Wildlife Service's fish monitoring activities show that stations which have reported high concentrations in the past continue to do so, and that the higher concentrations are associated with river systems having significant industrial activity. Such results are consistent with those for water and sediment from the USGS water quality file. The fish data, however, shows a decline in both the proportion of composites with some PCB residues and the proportion with residues in excess of 0.5 ppm in the 1970-73 sampling program. Levels of detection are generally lower in 1973 than in previous years. While there are cautionary notes (see section 2.1.5), some improvement in PCB contamination appears evident, although mostly at those stations with prior low contamination levels.

Water is recognized as being a major sink and transport mechanism for PCB's and, therefore, residue levels in water and fish are significant. Yet other media confirm the stability and breadth of contamination over the nation. Data on human adipose tissue show a steady climb over the years 1971-74 in the percent showing traces of PCB's. Soil monitoring shows that sixty percent, i.e., 3 of 5 cities sampled each year since the National Soils Monitoring Program started have had at least one positive PCB sample. Bird monitoring conducted as part of the U.S. Bureau of Fish and Wildlife's portion of the National Pesticides Monitoring Program shows residue levels in black ducks and mallards increasing between 1969 and 1972 in the Atlantic and Mississippi flyways and slightly decreasing in the Central and Pacific Flyways. PCB residues in starlings for 1970, 1972 and 1974 appear to be decreasing, yet PCB residues have been found in all samples each year, underscoring the ubiquitous nature of PCB's throughout the country.

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16. ABSTRACT

Review of the current PCB data base to assess the PCB levels in the environment on a national level; the full spectrum of PCB levels reported in man and the environment were of interest. Data were obtained from a number of nattional monitoring programs, the literature and many unpublished reports up to December 1, 1975.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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APPENDIX D

REVIEW OF CARCINOGENIC AND CO-CARCINOGENIC EFFECTS OF PCBs

D.1. The Experiment of Kimbrough *et al.* (266) on Female Sherman Rats.

This experiment followed a sequence of studies of the effects of PCBs on rats involving exposures lasting up to one year (179, 187, 189, 208). The results of these studies have been summarized in Sections III.9.1 and III.10.8 above, and were further summarized by Kimbrough (5) as follows:

> Dietary exposure of rats to the PCB's produces enlargement of the liver cells in some adult rats at a dietary level of 5 ppm (0.4 mg/kg/day) (ref. 3). Liver weights were increased in 21-day-old F_1 male weanlings when the dams were fed as little as 1 ppm of Aroclor 1254 (ref. 3). At a dietary level of 20 ppm or above of Aroclor 1254 or 1260, which is equivalent to a dietary intake of 1.5 mg/kg/body weight per day, for an extended period of time, the hepatocytes in many rats had foamy cytoplasm consistent with lipid accumulation. In addition, hyperchromatic nuclei and binucleation was observed in many liver cells. Inclusions were present in the cyptoplasm of the liver cells. These inclusions on electron microscopic examination were consistent with concentrically arranged membranes surrounding lipid vacuoles. Other ultrastructural changes observed at this and higher dietary levels in rats consisted of an increase in smooth endoplasmic reticulum and atypical mytochondria. These ultrastructural changes have also been described in primates (ref. 4) and are known to occur with a number of other xenobiotics. Similar observations were made in a study with smaller numbers of animals where the rats were fed Aroclor 1242 and Aroclor 1016 (ref. 5).
>
> A brown pigment representing ceroid pigment and uroporphyrin was observed in Kupffer cells and macrophages. Particularly at higher dietary levels, livers

showed pink fluorescence under UV lights.

In addition, exposure of rats and mice to PCB's for 6 months or longer produced grayish white lesions in the liver. On microscopic examination, these lesions consisted of proliferation of glandular epithelial cells that formed ducts and were surrounded by very pronounced fibrosis (refs. 6,7). The ducts often contained cellular debris. This lesion, called adenofibrosis, was first described by Edwards and White (ref. 6) in rats fed the carcinogen butter yellow (P-dimethylaminoazobenzene).

When rats were exposed for 6 months to a dietary intake of 500 ppm Aroclor 1254 and then sacrificed first at monthly and then at bimonthly intervals, the adenofibrosis persisted through a 10-month observation period. Usually adenofibrosis in rodents occurs concomitantly with hepatocellular carcinomas and/or neoplastic nodules (hyperplastic nodules). This was true for the Balb/cJ mouse (ref. 7).

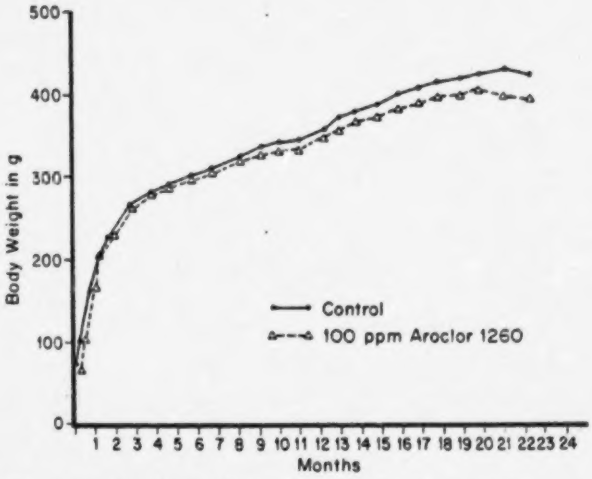
[Refs. 3-7 in this passage are the same as refs. 179, 267, 208, 268 and 189 respectively in this document.]

In this experiment (266) 400 weanling Sherman strain COBS female rats 21-26 days old were divided at random into two groups: 200 were maintained as controls and 200 were fed ad libitum a diet containing a nominal concentration of 100 ppm Aroclor 1260 (Lot No. AK-3, which contains 0.8 ppm of PCDFs, according to ref. 32). Measured concentrations of PCBs ranged from 70-107 ppm in the treated diet and less than 0.1 ppm in the control diet. Aflatoxins were not detectable in either diet. Based on measured food consumption, intake of PCBs declined from 11.6 mg/kg/day during the first week of exposure to 6.1 mg/kg/day at 3 months of exposure and to 4.3 mg/kg/day at 20 months (see Text-Figure 2 below). Exposure was continued until the animals were 21.5 months old; after six weeks on an uncontaminated diet (i.e., at the age of 23 months) all surviving animals were killed and autopsied. Although the treated rats grew significantly more slowly than the controls (Text-Figure 1 below), survival was good in both groups (173/200 controls, 179/200 treated).

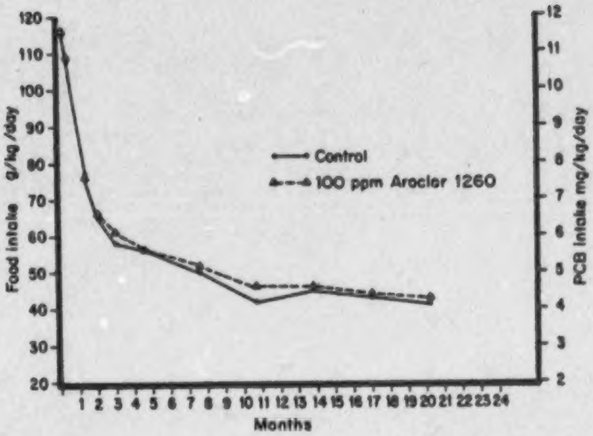
The pathological findings were reported and discussed by the authors as follows:

Pathologic Findings

Control rats (173) and experimental animals (184) were examined grossly and microscopically. The experimental group included 5 rats that were killed 1-2 months before the final kill. The remaining animals



TEXT-FIGURE 1.—Average body weight of control rats and those consuming Aroclor 1260.



TEXT-FIGURE 2.—Food intake of control and experimental rats and consumption of Aroclor 1260 by experimental rats.

were not included because of improper tissue fixation or because they died early in the experiment.

A consistent difference in the appearance of the livers was observed between the experimental and control groups. Almost all (170/184) livers of the experimental animals had from a few to multiple elevated tan nodules on the surfaces; additional nodules were usually seen on sectioning. These nodules varied from 0.1 to several cm in diameter, and in some rats replaced almost the entire liver. In contrast, the liver of only one control showed gross abnormalities and was markedly enlarged, nodular, tan, and firm. In addition, a variety of tumors of other organs was observed in both the experimental and the control groups. The incidence and type of tumors are given in table 1.

Histologic examination showed that 26 experimental animals and 1 control with enlarged, nodular livers had hepatocellular carcinomas. The additional 144 experimental rats with gross liver nodularity had hepatocellular nodules characteristic of neoplastic nodules [synonym, "hyperplastic nodules" (*9*)]. A recent workshop sponsored by The National Cancer Institute[3] recommended the term "neoplastic nodules" for these lesions as a more accurate indication of their biologic significance. No nodules were in control animals.

The hepatocellular carcinomas were well-differentiated trabecular types (figs. 1–3), except for three in the experimental animals which had a glandular, papillary pattern (fig. 4). The trabecular tumors showed severe disruption of normal liver architecture and were usually easily recognized at low magnification. Liver plates, two or more cells thick in some areas, were arranged in haphazard linear, branching, or pseudoglandular patterns. Different patterns were usually present in the same tumor. Blunt-ended plates, sinusoidal ectasia, and congestion were frequent. The hepatocytes varied from a normal appearance to enlarged, acidophilic, or diffusely basophilic cells with large, hyperchromatic nuclei and prominent nucleoli. The cytoplasm often contained eosinophilic inclusions within vacuoles. Mitotic figures were sometimes present. Foci of coagulative necrosis were occasionally observed in cancerous areas, but there

TABLE 1.—*Incidence and type of liver lesions and tumors of other organs examined histologically*

Organ or tissue	Type of lesion	Incidence	
		Controls	Experimental
Liver	Hepatocellular carcinoma	1/173	26/184
	Neoplastic nodules	0/173	144/184
	Foci or areas of cytoplasmic alteration	28/173	182/184
Thyroid gland	Parafollicular cell tumor	37/160	18/166
Adrenal gland	Pheochromocytoma	1/173	1/167
Pituitary gland	Chromophobe adenoma	41/153	28/139
	Carcinoma	0/153	1/139
Uterus	Endometrial polyp	18/149	25/163
	Adenocarcinoma	0/149	2/163
	Sarcoma of endometrial stroma	3/149	7/163
Urinary bladder	Transitional cell papilloma	1/167	0/169
Mammary gland	Fibroadenoma	17/173[a]	13/184[a]
	Adenocarcinoma	5/173[a]	1/184[a]
Salivary gland	Fibrosarcoma	1/173[a]	0/184[a]
Lung	Adenoma	2/173	2/184
Adipose tissue	Lipoma	0/173[a]	2/184[a]
Brain	Glioma	0/173	2/184
Ovary	Granulosa theca cell tumor	5/149	0/163
	Papillary adenoma	1/149	2/163
Hematopoietic system	Granulocytic leukemia	1/173	0/184
	Lymphoma	0/173	2/184
Kidney	Hemangioma	0/173	1/184
Thymus	Thymoma	1/173[a]	0/184[a]
Parathyroid gland	Adenoma	0/173	2/184
Skin	Fibroma	0/173[a]	1/184[a]

[a] Incidence based on gross detection with microscopic confirmation.

was no fibrosis or other evidence of chronic degenerative changes. Periodic acid-Schiff (PAS) without diastase stained carcinomas less intensely than uninvolved liver, which suggested a decrease in glycogen. Most carcinomas were more basophilic than the normal liver with azure eosin stain. No definite intravascular invasion or metastases were found.

Neoplastic nodules were generally spherical and well demarcated, and occupied areas equal to those of several liver lobules (fig. 5). The cells in these nodules were generally enlarged, and the cytoplasm was either ground-glass-appearing, diffusely basophilic, or clear. Enlarged hyperchromatic nuclei, double nuclei, and mitotic figures were often present. The cytoplasm frequently contained inclusions similar to those in the carcinomas, except they were larger and appeared as whorled, concentric lamellae. In previous studies (*3*) these formations were shown by electron microscopy to represent aggregates of smooth endoplasmic reticulum. The normal liver architecture was absent within nodules, and cells were in sheets or irregular plates. Portal areas and central veins were absent and sinusoids were dilated in some areas. At the periphery of the nodules, the surrounding liver plates were tangentially arranged and narrowed, due to compression (figs. 6, 7). Nodules varied in PAS positivity, but always differed from surrounding liver. Most nodules were more eosinophilic, and a few were more basophilic than the normal liver with azure eosin stain.

In 182 treated and 28 control animals, there were also foci or areas of hepatocytes with altered cytoplasm (figs. 8–10). In controls, these were mostly collections of cells with water-clear cytoplasm. In treated animals, most affected cells were enlarged and had eosinophilic, ground-glass-appearing cytoplasm or were diffusely basophilic and smaller than normal cells. In basophilic areas, sinusoids were dilated and liver plates somewhat tortuous. In general, the cells in these areas were like those in neoplastic nodules, but there were no architectural alterations, and plates of involved liver cells merged with the surrounding liver.

No unusual features were noted in tumors of other organs, and there were no apparent differences in incidence between experimental and control animals. Parafollicular thyroid tumors varied from small circumscribed nodules of pale, oval-to-spindle cells to masses that obliterated the gland and invaded the capsule. They were similar to those described by Boorman et al. (*10*). Other frequent tumors included pituitary chromophobe adenomas, mammary fibroadenomas, and endometrial polyps. Endometrial stromal sarcomas were also present in 10 animals.

The only bladder tumor observed was in a control animal. Therefore, the occurrence of the bladder tumor in the previous experiment was apparently unrelated to the ingestion of Aroclor 1260.

A few rats in the experimental group also showed areas of adenofibrosis (synonym, "cholangiofibrosis") of the liver, a lesion described previously following the administration of Aroclor 1260 (*4*).

DISCUSSION

The livers of treated animals showed neoplastic lesions in 170 of the 184 examined, and in only 1 of 173 controls. Although only 26 of the lesions in treated rats were clearly carcinomas according to traditional histologic criteria, neoplastic nodules are part of the spectrum of response to hepatocarcinogens and must be included in the evaluation of tumorigenesis.

In the past, these liver lesions have been interpreted in various ways, and different names have been used to report them: nodular hyperplasia, hyperplastic nodules (*11*), hepatomas (*12, 13*), and hepatic nodules (*14*). Similar ambiguity exists in the classification of human liver lesions of this type (*15*).

Several studies with known carcinogens have demonstrated the development of liver nodules, indistinguishable from the neoplastic nodules in this study, before the appearance of carcinoma (*11, 16–18*). In a recent review (*9*), the biology and significance of nodules and their relationship to hepatocellular carcinoma were thoroughly discussed. In our study, Aroclor 1260 induced a spectrum of nodules and cancers in treated animals as outlined by the Rat Liver Tumor Workshop.[9]

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[9] Rat Liver Tumor Workshop at Silver Spring, Md., Dec. 11–13, 1974; sponsored by Carcinogenesis, Division of Cancer Cause and Prevention, National Cancer Institute.

Comments. This was a well-designed and well-conducted study, deviating from recommended protocols in only one respect: its limitation to female rats. (In earlier studies by the same authors, males appeared as sensitive or more sensitive than females to 100 ppm Aroclor 1260, as measured by the degree of increase in liver weight -- Tables III.9.2 and III.9.3). Two other limitations may be noted: (a) the rats were not exposed prior to weaning, although the authors'

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own studies (Table III.9.3) had indicated that rats exposed to PCBs during gestation and nursing show early liver changes at much lower dietary levels; (b) the rats were killed at 23 months, relatively early in comparison to the 26-30 month expected life span. Each of these design features of the experiment, although common practice in carcinogenesis bioassays, is likely to have reduced its sensitivity in demonstrating carcinogenic effects; the early termination is likely to have arrested tumors at a relatively early stage of development.

Survival of the rats was very good; the only gross indication of toxic effects was the reduced growth rate, although Aroclor 1260 is known to have other physiological and biochemical effects in rats at 100 ppm. The liver tumors and nodules were well described, well illustrated, and related to current understanding of liver pathology in rats (269). Indeed, several of the liver slides from this study were examined by a panel of expert pathologists at the workshop where the terminology used in the paper was developed (269). The only unusual aspect of the study was the finding of one hepatocellular carcinoma in an untreated rat.

This study thus provides good evidence for the hepatocarcinogenicity of Aroclor 1260 in female rats. Because only one rather high dose was used, and because the rats were exposed for less than 21 months, however, the results provide little quantitative information on dose-response relationships.

Among other tumors listed in the authors' Table 1, the only type showing a statistically significant change in incidence is the parafollicular cell tumor of the thyroid gland, whose incidence was reduced from 37/160 to 18/166 ($P < 0.01$). It is not clear whether this reduction should be attributed to chance occurrence among the 17 organs examined, or whether it should be related to the known effects of PCBs on thyroid function (Section III.12.3).

D-2 Industrial Bio-Test Experiment with Charles River Rats.

This experiment was described in an unpublished report dated November 1971 (207) and a brief summary of results was presented at the National Conference on Polychlorinated Biphenyls in November 1975 (68). One thousand Charles River strain albino rats were divided into 10 treatment groups. 100 rats (50 male and 50 female) served as common controls; 100 rats (50 male and 50 female) were included in each of nine exposed groups, fed diets containing 1, 10 and 100 ppm of Aroclors 1242, 1254 and 1260 respectively. Dosage started when the animals were about 4-6 weeks old (mean weights 133-136 g) and continued for 24 months. However, 5 males and 5 females were sacrificed from each treatment group for interim studies after 3, 6 and 12 months, so that each treatment/sex subgroup contained only 35 animals at the start of the second year. Mortality was high, especially in the animals fed 100 ppm (Table D1), so that only 6-21 animals from each treatment/sex subgroup survived to the terminal sacrifice at 24 months (i.e., when the animals were about 25 months old). The original report gives summaries of body weights, food consumption, clinical chemistry, organ weights, and histopathological diagnoses for each of the 20 subgroups. As in the experiment of Kimbrough et al., the principal effects were noted in the liver and in tumor incidence: these are summarized from the original report (207) in Tables D1 and D2.

Table D1 summarizes data on survival, liver weight, and tumor incidence. Liver weights were significantly increased in the animals fed 100 ppm (except the males fed Aroclor 1242); the effect was largest with Aroclor 1254. The incidence of tumors was similar in all groups, except for chromophobe adenomas of the pituitary, which were not found in controls but were found in 8 of the 9 treated groups and in 12 of the 18 subgroups. The number of surviving animals was too small to compare individual groups with controls, but if all the treated animals are considered together the increased incidence approaches statistical significance. Almost all of these pituitary tumors were found in the animals killed at 24 months, but 3 were found in animals dying at 20-23 months.

Table D2 summarizes the histopathologic findings reported in the original report (207) for the livers of control animals and those treated at 100 ppm. These diagnoses were summarized by the original pathologist as follows:

> Significant liver injury was found in animals fed 100 ppm Aroclor 1260 (T-III). The compound associated lesions consisted of vacuolar change, focal hypertrophy and focal hyperplasia. In addition, there were lesions of inflammation, necrosis, fibrosis and minor degeneration in this group which, although seen in the controls and lower test groups, were more severe and frequent in the T-III group.

Table D1. Industrial Bio-Test Rat Experim[illegible]: Basic Results.

Group	Sex	No. at 12 mo.	Survival to month			Liver % of body wt.24 mo.	Animals with tumors at 20-24 months			
			18	21	24		Abdomen & Mammary	Pituitary	Others	TOTAL
CONTROL	M	35	25	19	10	3.47	1	0	1	2
	F	35	26	19	16	3.05	6	0	2	7
Aroclor 1242										
1 ppm	M	35	25	18	10	2.93	2	0	1	3
	F	35	28	24	10	3.00	10	2	2	11
10 ppm	M	35	25	20	12	3.18	2	1	1	3
	F	35	29	24	19	3.19	7	2	2	10
100 ppm	M	35	23	16	6	3.95	0	0	1	1
	F	35	27	19	10	4.82**	1	1	2	4
Aroclor 1254										
1 ppm	M	35	22	14	5	2.99	0	1	1	2
	F	35	29	24	21	3.10	11	1	2	11
10 ppm	M	35	26	18	8	3.21	0	0	2	2
	F	35	24	20	13	3.84*	6	2	2	9
100 ppm	M	35	24	17	8	4.75**	2	0	0	2
	F	35	22	18	12	8.62**	10	0	2	12
Aroclor 1260										
1 ppm	M	35	22	17	9	3.40	0	1	0	1
	F	35	27	21	16	3.35	5	3	1	6
10 ppm	M	35	20	12	6	2.87*	0	0	0	0
	F	35	26	21	18	3.19	5	3	3	8
100 ppm	M	35	22	16	6	4.67**	0	1	2	3
	F	35	23	16	12	4.42**	4	1	3	8

* $P < 0.05$, ** $P < 0.01$, significant difference from controls.

Table D2. Liver pathology reported in Industrial Bio-Test Rat Experiment: Controls and animals fed 100 ppm PCBs.

	Controls		Aroclor 1242 100 ppm		Aroclor 1254 100 ppm		Aroclor 1260 100 ppm	
	M	F	M	F	M	F	M	F
REPORT OF NOVEMBER 1971 (ref. 207)								
Animals surviving 24 months	10	16	6	10	8	12	6	12
Animals with diagnoses reported at "final sacrifice":	11	14	6	14	11	15	10	15
Focal lymphoid infiltration	-	3	-	-	1	-	-	-
Focal degeneration	1	-	1	3	2	-	1	-
Necrosis	-	-	-	-	-	-	-	2
Vacuolar change (fatty)	-	-	3	11	3	10	2	3
Hypertrophy[a]	-	-	2	5	3	6	2	5
Hyperplasia[b]	2	2	0	3	3	3	-	6
Nodular hyperplasia	-	-	-	1	-	1	-	-
Hepatoma	-	-	-	-	-	1	-	-
Miscellaneous[c]	-	1	-	2	1	-	4	2
REPORT OF NOVEMBER 1975 (ref. 68)								
Animals with diagnoses reported at "24 month sacrifice":	-		20		27		27	
Nodular hyperplasia	-		8		13		7	
Hepatoma	-		2		4		5	
Cholangiohepatoma	-		1		2		2	

---

[a] Includes focal and centrolobular hypertrophy. [b] Includes ductile and focal hyperplasia. [c] Focal hepatitis 2; focal cholengiohepatitis 1; portal fibrosis 2, congestion 3, telangiectasis 1, chronic inflammation 1.

> The vacuolar change was not seen in the control animals but was observed occasionally in livers from T-I (1 ppm) and T-II (10 ppm) animals. It was frequent in the T-III animals. This lesion is morphologically indicative of fatty degeneration. Formalin-frozen sections of livers from representative animals which displayed vacuolar changes were stained with Oil Red O to reveal the presence of fat. The vacuolar lesion in the cytoplasm of these cells was positively identified as fat.
>
> The hypertrophic change found in the liver was focal and often limited to the central lobular area where groups of cells were swollen to two or three times their normal size with clear pink homogenous cytoplasm. The hyperplasia was associated with the same cells and appeared to be an extension of the hypertrophic lesion. The hyperplastic cells were also usually hypertrophic. The most severe examples of hyperplasia appeared as nodular growths with limited compression of the surrounding normal hepatic tissue.
>
> The minor lesions of degeneration, hepatitis, ductule cell proliferation, necrosis and focal lymphoid infiltration seen in the control animals and the T-I and T-II groups are lesions of spontaneous disease and are not related to the Aroclor 1254. This level of liver disease is not unusual in rats of this age. (207, pp. 67-68.)

In 1975 the liver slides were re-examined by the original pathologist (416). Revised diagnoses are tabulated in Table D2A and summarised in the second part of Table D. Although the 1975 report states that "in most instances, the spectrum of treatment-related histopathological findings in the liver from this re-evaluation did not differ significantly from that previously reported in our original report dated November 12, 1971" (416, p. 2), the diagnoses were in fact markedly different, including 11 hepatomas, 5 cholangio-hepatomas, and 28 examples of nodular hyperplasia in the animals treated with 100 ppm.

Table D2A. Liver pathology reported in Industrial Bio-Test rat experiment: 1975 re-diagnosis (ref. 416).

	Control	Aroclor 1242			Aroclor 1254			Aroclor 1260		
Dose rate (ppm):		1	10	100	1	10	100	1	10	100
No. of animals	23	31	30	20	31	26	27	25	23	27
Vacuolar change	1	7	8	9	8	10	13	5	6	10
Focal hypertrophy	0	2	3	8	3	5	13	3	10	11
Nodular hyperplasia	1	0	2	8	0	3	13	0	9	7
Hepatoma	0	0	0	2	0	0	4	0	0	5
Ductular hyperplasia	5	3	3	3	6	3	4	6	5	14
Cholangio-hepatoma	0	0	0	1	0	0	2	0	0	2
Hepatocellular necrosis	1	1	1	0	3	1	2	4	2	6

Nodular hyperplasia was also reported in 14 animals treated with 10 ppm and in one control. The tumors were described as follows:

> "Those lesions classified as hepatomas or cholangiohepatomas were larger nodules which showed evidence of confluence or some variation in cell size, shape, staining, or a ductular or adenomatous pattern of growth. In the absence of metastasis, invasiveness, severe basophilia, mitoses, or other evidence of anaplasia, these are benign tumors rather than malignant tumors (carcinomas)." (416, p. 3)

There are substantial discrepancies between the total number of animals diagnosed and the numbers listed as surviving to 24 months in the original report (Table D2).

Comment. Although this experiment was continued for a full 24-month period and provides a useful comparison between the three Aroclors, the experimental groups were too small to provide a sensitive test for carcinogenicity. In particular, the use of only a single control group, reduced to 70 animals by interim sacrifices and to 26 by mortality in the second year, made the entire experiment very insensitive. The cause of the high mortality was not stated. The drastic changes in diagnoses between 1971 and 1975 were not explained and the tumors were inadequately described.

The 1975 report indicates that PCBs at 100 ppm in the diet induced a variety of proliferative lesions, including tumors and hyperplastic nodules, in the livers of exposed rats, and that these effects were induced at roughly similar frequency by each of the three Aroclors. No further conclusions can be drawn without a complete report and independent diagnoses of the lesions in the livers and pituitaries.

D.3 Apparent Conflict Between Results of Kimbrough and Industrial Bio-Test Experiments.

In presenting the revised results of the Industrial Bio-Test experiment, Calandra (68) suggested that they conflicted substantially with those of Kimbrough *et al*. Specifically, he emphasized that no hepatocellular carcinomas could be diagnosed in the Industrial Bio-Test experiment, that two consultants (in addition to the original pathologist) had reviewed the slides in 1975, and that one of these consultants had also reviewed slides from the Kimbrough, *et al*. experiment and "does not agree with the reported findings". However, this consultant also disagreed with the Industrial Bio-Test pathologists, reporting no tumors or even hyperplastic nodules in that experiment (417). In his review of the slides from the Kimbrough *et al*. experiment, this consultant diagnosed among 184 treated animals 136 hyperplastic nodules and 43 "hyperplastic nodules with atypical structures" (Table D2B). A review of this report suggests that the dispute is primarily over terminology, in particular over the severity of the criteria required for a diagnosis of neoplasia. This is an area in which the Agency has considerable expertise and has previously accepted the criteria and diagnoses of the principal pathologist* in the Kimbrough *et al*. study (270). Recognizing the terminological dispute, there does not seem to be substantial qualitative conflict between these two experiments. Even without the supporting evidence cited below, the two experiments together would justify a finding that Aroclor mixtures induce neoplastic transformations in rat livers.

---

* Dr. R. A. Squire, Head of the Tumor Pathology Section of the National Cancer Institute.

Table D2B (from ref. 417)

Differences of liver lesions in control and experimental rats treated with Aroclor 1260

	Experimentals	Controls
Vacuolization	155	13
Focal granular alteration	100	31
Single granular alteration	169	45
Single cell necrosis	125	17
Group cell necrosis	36	2
Cell enlargement	175	17
Ductal proliferation	52	3
Cholangiomatous lesion	7	-
Stern cell proliferation	121	87
Hyperplastic nodules:		
without atypia	136	9
with atypia	43	1

D.4 52-week Study by Ito et al. in Rats.

Ito et al. (190) reported a study involving 290 male Wistar rats. The sizes of the experimental groups were not precisely reported but it appears that 20 rats were used as a common control and 30 were included in each of 9 experimental groups, dosed with 100, 500, and 1000 ppm of Kanechlors 300, 400 and 500. Exposure started at 8 weeks of age and the animals were killed after 28 to 52 weeks. The results of the study are summarized in Tables D3 and D4. There were marked increases in liver weight in all treated groups and apparently high mortality in the groups fed 500 to 1000 ppm. Histopathologic changes included bile duct proliferation, cholangiofibrosis and nodular hyperplasia (Table D4). The nodular hyperplasia is not described in detail but is said to have been similar to that induced by other carcinogens. The cholangiofibrosis was apparently similar to that reported by Kimbrough et al. (191, 266): its possible relationship to carcinogenesis is discussed above.

Comment. This study was conducted for far too short a period to serve as an adequate carcinogenicity test: other defects included the small size of the control groups, the high doses used, high mortality, and inadequate description of the lesions. However, it suggests that all three Kanechlors tested induce pre-neoplastic lesions in rats exposed to high doses.

D.5 The Study of Kimura and Baba on Donryu Rats.

Kimura and Baba (271) conducted a short-term experiment in rats of the Donryu strain. Starting at 10 weeks of age, 10 males and

Tables D3 and D4 (from ref. 190)

N. ITO, ET AL.

Changes in Body and Liver Weights of Male Wistar Rats Given Polychlorinated Biphenyls (PCB)

PCB in diet (ppm)		Experimental period (weeks)	Effective No. of rats	Body weight (g)		Liver weight	
				Initial	Final	(g)	% of body wt.
Kanechlor-500	(1000)	49.3	13	126.4±7.6*	288.2±65.7	22.2±3.3	8.0±1.1
	(500)	52.0	16	122.5±8.4	378.4±38.2	19.5±2.5	5.2±0.6
	(100)	52.0	25	124.8±8.2	469.6±72.7	17.3±2.1	3.7±0.6
Kanechlor-400	(1000)	40.0	10	163.0±7.8	199.6±42.8	16.7±5.4	8.4±2.3
	(500)	27.8	8	188.0±6.6	209.5±43.6	12.8±2.0	6.2±1.1
	(100)	39.6	16	175.0±18.8	401.8±121.9	14.4±3.4	3.6±0.7
Kanechlor-300	(1000)	52.0	15	128.3±9.0	420.8±56.5	18.4±3.0	4.3±0.4
	(500)	52.0	19	135.4±7.2	457.5±60.7	18.8±3.3	4.1±0.4
	(100)	52.0	22	125.2±8.5	506.3±65.4	14.8±2.1	2.9±0.2
Control		52.0	18	130.6±4.6	553.3±69.6	13.0±1.9	2.4±0.2

* Values are mean ± SD

Histopathological Findings in the Liver of Male Wistar Rats Given Polychlorinated Biphenyls (PCB)

PCB in diet (ppm)	Histopathology of the liver							
	Oval cell proliferation	Bile duct proliferation	Fatty changes	Cell hypertrophy	Fibrosis	Cholangiofibrosis (%)	Nodular hyperplasia (%)	Amyloidosis (%)
Kanechlor-500 (1000)	+	+	+	⧺	±	4/13 (30.8)	5/13 (38.5)	0/13 —
(500)	±	+	+	+	—	0/16 —	5/16 (31.3)	0/16 —
(100)	±	±	±	±	—	0/25 —	3/25 (12.0)	0/25 —
Kanechlor-400 (1000)	+	+	±	⧺	⧺	2/10 (20.0)	3/10 (30.0)	0/10 —
(500)	±	±	—	+	—	0/8 —	0/8 —	0/3 —
(100)	±	±	—	+	—	0/16 —	2/16 (12.5)	2/16 (12.5)
Kanechlor-300 (1000)	+	+	+	+	—	2/15 (13.3)	0/15 (6.7)	0/15 —
(500)	—	±	±	+	—	0/19 —	0/19 —	0/19 —
(100)	—	±	±	±	—	0/22 —	1/22 (4.5)	0/22 —
Control	—	—	—	—	—	0/18 —	0/18 —	0/18 —

10 females were exposed to Kanechlor-400 at dietary levels varying from 38.5 to 616 ppm (for dosage regime see foot of Table D5); 5 males and 5 females served as controls. The treated animals lost weight, and suffered from lung abscesses, pneumonia, spleen atrophy, and intracranial abscesses, which the authors suggest may have been due to a treatment-related loss in resistance to infection. "Multiple adenomatous nodules", which the authors interpreted as a "benign neoplastic lesion...still short of malignancy" were found in the treated females (Table D5).

Comments. In view of the small number of animals used, the high dosage, the animals' poor condition, and the early termination of the experiment, this study can be regarded as no more than a preliminary screening test. However, it did provide evidence of early neoplastic changes, but these were not described and cannot be evaluated.

D.6 The Experiment of Kimbrough and Linder on BALB/cJ Mice.

Kimbrough and Linder (191) reported an experiment in which two groups of 50 BALB/cJ male mice were fed 300 ppm of Aroclor 1254 in the diet starting at the age of 5-6 weeks. One group was exposed for 11 months; the other was fed for six months, followed by five months on untreated diet; two groups of 50 male mice served as controls. Mortality was high early in the experiment due to fighting, but there was still excess mortality in the treated groups after the mice were recaged (34/80 versus 19/77 in the two control groups). The results are summarized in Table D6. The treated mice showed gross enlargement of the liver, including 11 hepatomas and adenofibrosis in all 22 animals

Table D5 (from ref. 271)

N. T. KIMURA AND T. BABA

Pathological Findings Induced by Long-term Oral Administration of Kanechlor-400 in Rats

Rat No.	Sex	Total exp. period (days)	Approx. amount of KC-400 ingested (mg)	Body weight (g)		Liver			Other pathological findings*
				Initial	Final	Ratio to body wt. (%)	Fatty degeneration	Multiple adenomatous nodules	
1	♂	159	450	199	309	4.1	+	−	SA, Pn, LA
2	♂	256	900	192	273	5.4	+	−	
3	♂	323	1200	201	192	6.8	+	−	SA, Pn, FDAd
4	♂	339	1300	204	199	6.8	+	−	Pn, D, FDAd
5	♂	347	1300	200	328	6.2	+	−	Pn, LA
6	♂	353	1300	230	210	5.4	+	−	SA, Pn, IA
7	♂	355	1300	201	296	5.2	+	−	Pn
8	♂	357	1300	185	226	6.3	+	−	Pn, LA, FDAd
9	♂	373	1300	234	325	6.5	+	−	LA
10	♂	530	1800	228	297	5.8	+	−	LA
11	♀	244	700	158	137	5.9	+	−	LA
12	♀	357	1100	161	184	6.2	+	−	Pn, D
13	♀	366	1100	154	177	7.1	+	−	LA, D
14	♀	396	1100	165	125	7.8	+	−	Pn, LA, D
15	♀	402	1200	202	212	6.7	+	+	LA, D
16	♀	461	1500	168	148	8.4	+	+	LA, D, IA, FDAd
17	♀	477	1500	178	165	7.8	+	+	LA, D, IA, SA,
18	♀	482	1500	225	145	6.3	+	+	LA, IA, SA
19	♀	504	1500	191	167	6.5	+	+	LA, D, FN
20	♀	560	1500	209	253	6.4	+	+	D, AAd
21	♂	255		257	471	2.5	−	−	FDAd
22	♂	332		140	432	3.1	−	−	Pn, FDAd
23	♂	538		190	426	2.7	−	−	FDAd
24	♂	538		182	523	2.5	−	−	
25	♂	538		256	456	2.7	−	−	FDAd
26	♀	331		192	198	4.2	+	−	Pn
27	♀	448		192	495	2.6	+	−	Pn
28	♀	538		202	292	2.9	−	−	
29	♀	538		201	320	3.0	−	−	
30	♀	538		193	314	2.6	−	−	

* SA = spleen atrophy, Pn = pneumonia, LA = lung abscess, FDAd = fatty degeneration of the adrenal cortex, D = depilation, IA = intracranial abscess, FN = extensive fat necrosis in the abdomen, AAd = adenoma of the adrenal cortex.

Schedule of feeding:

Concn. of Kanechlor-400 (ppm)	38.5	77	154	308	616	462	——	462	——	462	
Period of feeding (days)	26	57	21	21	56	39	28	98	28	82	Total 400

A pause (——) in PCB feeding was inserted similarly in experimental and control groups. during which and after termination of diet feeding, the rats had free access to the conventional diet (CE-2).

.—*Body weights, liver weights, and hepatoma incidence in surviving mice fed Aroclor 1254 in the diet and in controls fed plain chow*

Dietary level (ppm)	Dosage level (mg/kg/day)	Time exposed to experimental diet	Total mice	Total survivors	Final body weight of survivors: arithmetic mean (g)	Liver weight: arithmetic mean (g)	Liver weight expressed as percentage of body weight	Hepatoma incidence* in survivors
0	0	0	50	34	30. 7	1. 77	5. 8	0
0	0	0	50	24	30. 9	1. 78	5. 8	0
300	49.8	11 months	50	22	33. 8†	8. 62†	25. 5†	3(0. 1–0. 4 cm ø) 3(0. 5 cm ø) 4(1 1. 5 cm ø)
300	Not checked	6 months and 5 months' recovery.	50	24	31. 5	2. 37†	7. 5†	1(0. 3 cm ø)

*Determined when mice were 12 months old. ø=diameter.

†$P<0.001$. A *t*-test was used for the statistical evaluation.

Table D6 (from ref. 191)

fed for 11 months. The pathological changes were illustrated (191, Figures 1-5) and described and discussed as follows:

> Microscopic examination of the livers of the 22 survivors in the group receiving the experimental diet for 11 months (fig. 1) showed markedly enlarged liver cells. The nuclei of the hepatocytes were enlarged, hyperchromatic, and atypical. The cytoplasm was either smooth or vacuolated. Macrophages and Kupffer's cells contained a brown pigment. Slight, diffuse interstitial fibrosis and numerous, large extracellular hyaline bodies were also seen. Some livers had extensive areas of coagulation necrosis and fibrosis (fig. 2). A general pleomorphism was noted in a number of livers. Degeneration, necrosis, and atrophy of individual liver cells were also observed.
>
> A few nuclei showed karyorrhexis. Some smaller cells and proliferation of the focal nodular type were also noted. The irregular outer surface of the liver with areas of retraction indicated that proliferation, atrophy, and regression had occurred. Occasional calcareous deposits ≈5–8μ in diameter were present. In each of the livers, several areas were seen where the parenchyma was replaced by glandular formations of proliferated epithelial cells which formed ducts and often produced mucus. These epithelial cells were surrounded by either sparse (fig. 3) or abundant connective tissue.
>
> In addition to the above findings for all 22 livers of mice treated for 11 months (table 1), 10 hepatomas were found (fig. 4); of these, 3 measured 0.1–0.4 cm in diameter, 3 measured 0.5 cm, and 4 measured 1–1.5 cm. The tumors developed in a total of 9 mice. On gross inspection, they appeared as tan nodules. On microscopic examination, they consisted of well-differentiated hepatocytes that were relatively uniform and usually smaller than the surrounding liver cells. The tumors were well circumscribed and surrounded by compressed hepatic parenchyma or strands of fibrous tissue. The larger tumors contained areas where the sinusoids were dilated and filled with a pink-staining amorphous material. On gross inspection of the organs at autopsy, metastases were not seen. However, detailed screening for metastases including serial sectioning of lungs was not undertaken.
>
> Most livers of the 24 mice surviving 6 months of dietary exposure to PCB's followed by a 5-month recovery had enlarged cells; often these liver cells had enlarged, atypical, hyperchromatic nuclei, although the livers at autopsy weighed only slightly more than those of the controls. Necrosis of individual liver cells was observed and some nuclei showed karyorrhexis.

The macrophages and Kupffer's cells usually contained a brown pigment. Many liver cells, particularly those in the periphery of the lobules, had vacuolated cytoplasm. The livers of about two-thirds of these mice showed slight-to-moderate diffuse, interstitial fibrosis. The outer surface of the livers still showed small indentations but not as pronounced as those in the group receiving PCB's for 11 months. Numerous extracellular hyaline bodies were observed in some livers. In addition, many calcareous deposits, light in the center and darker in the periphery, were also seen (fig. 5). They were more frequent in this group than in the one fed PCB's for 11 months. A few livers showed bile duct proliferation. Only 1 liver contained a small hepatoma measuring 0.3 cm in diameter. The liver cells comprising the hepatoma were well-differentiated hepatocytes.

Except for the subcutaneous abscess formations in some mice and 1 sweat gland adenoma, no other findings of note were made in the mice of any groups.

DISCUSSION

Two significant findings were made in the present study: adenofibrosis of the liver and hepatomas. Thus adenofibrosis can also be induced in mice and does not occur solely in rats where it has been described previously (*1*, *3*). A similar lesion may occur in hamsters (*8*). In the mouse lesion, the epithelial component was much better differentiated and less pleomorphic than in the rat lesion (*1*). The pattern of the lesions and the interrelationship with fibrosis were the same. Ducts and cysts formed in areas of adenofibrosis in both the mouse and rat livers. Whether adenofibrosis is a precursor of a malignant lesion or occurs concomitantly with malignant lesions of the liver is still debated. In an excellent review, Stewart and Snell (*4*) concluded that the lesion showed no convincing evidence of a precancerous lesion. Reuber (*8*) postulated that cholangiocarcinomas would develop from adenofibrosis in rats as well as hamsters.

The mouse strain used in this study only rarely develops hepatomas spontaneously. Peters et al. (*5*) observed 6 hepatomas in a group of 2000 BALB/cCr mice, a subline from the Andervont subline at the National Cancer Institute; one would expect incidences similar to those observed in the BALB/cJ subline. Madison et al. (*6*) found 13 (0.62%) hepatomas in 2088 BALB/cCr mice 18 months of age. In the present study none of the controls, 1 of 24 mice fed PCB's for 6 months, and 9 of 22 mice fed PCB's for 11 months developed hepatomas.

> Although the hepatomas were well differentiated, according to Andervont and Dunn (9), they represent potentially malignant lesions, a certain percentage of which can metastasize and be transplanted. From the appearance of the lesion, it is impossible to predict which tumor will be malignant. The size of the liver tumors cannot be used as a criterion. Quite possibly the tumors would have been larger and would have occurred more often had the mice been allowed to complete their lifespans.

Comments. This experiment deviated from accepted protocols for carcinogenesis bioassays primarily in its very early termination, and in being limited to males. The high mortality and the consequent small number of treated animals surviving to 11 months makes it further insensitive as more than a preliminary screening test for carcinogenicity. However, there was a significant incidence of liver tumors which, as the authors point out, are rare in control mice of this strain and are at least potentially malignant. These tumors were well described and well illustrated. Thus, despite its limitations, this experiment gives convincing evidence that Aroclor 1254 induces very early neoplastic changes in the mouse liver.

D7. The Experiment of Ito et al. with PCBs and BHC in dd Mice.

Ito et al. (192) reported an extensive series of experiments in which groups of 12-30 male dd mice were exposed to various levels of PCBs (Kanechlors KC-300, KC-400, KC-500 at 100-500 ppm in the diet) alone or in combination with one of three isomers of BHC (hexachlorocyclohexane). Two control groups contained only 6 and 20 male mice. Treatment started at the age of 8 weeks and the animals were killed and examined after 24 or 32 weeks.

Results of the experiment are summarized in Tables D7 and D8. Liver weights were markedly increased in all groups fed PCBs at all dose levels, but were only slightly increased in the groups fed isomers of BHC alone. KC-500 induced nodular hyperplasia and hepatocellular carcinoma in 12/12 mice within 32 weeks at the highest dose rate (500 ppm), but did not do so at lower dose levels; nor did KC-400 or KC-300 even at 500 ppm (Table D7). KC-500 at 250 ppm in the diet also markedly increased the carcinogenic effects of BHC (Table D8). Whereas BHC alone did not induce nodular hyperplasia or carcinomas within 24 weeks, except in the group fed α-BHC at 250 ppm, the groups fed α-BHC and β-BHC together with KC-500 developed both kinds of lesions within 24 weeks, even at a dietary level of 50 or 100 ppm BHC (Table D8). The hyperplastic nodules and carcinomas induced by PCBs were well illustrated (199, Figures 4-6) and were described as follows:

> *Histopathologic findings:* The histologic changes and incidence of nodular hyperplasias and hepatocellular carcinomas in the livers of animals in the various groups are given in table 4.
>
> Oval cell infiltration and bile-duct proliferations were rare in all groups. But hypertrophic changes of liver parenchymal cells in centrolobular areas were clear in groups that received α-BHC or β-BHC plus PCBs or α-BHC alone. Among the 30 mice in the group given 250 ppm α-BHC, nodular hyperplasia was found in 23 (76.7%) and hepatocellular carcinoma in 8 mice (26.7%). No hyperplastic nodules or hepatocellular carcinomas were seen in any other group fed α-BHC or β-BHC only. However, some mice in the groups that received 100 or 50 ppm α-BHC or 250 or 100 ppm β-BHC with PCBs developed nodular hyperplasias and well-differentiated hepatocellular carcinomas (fig. 6). The incidence of hepatocellular carcinoma in mice given 250 ppm α-BHC increased when the diet was supplemented with 250 ppm PCBs-5. No neoplastic changes were seen in groups fed α-BHC. The histologic patterns of nodular hyperplasias and hepatocellular carcinomas induced in mice by α-BHC or β-BHC were not affected by supplementation of the diet with PCBs. The histopathology of neoplasms induced by α-BHC or β-BHC was essentially the same as of that induced by PCBs in series I.

Table D7 (from ref. 199)

Histopathologic findings in the livers of male dd mice treated with technical polychlorinated biphenyls (PCBs) for 32 weeks

PCBs* in diet (ppm)	Histopathology of liver				Liver nodules (percent)	
	Oval cells	Bile-duct proliferation	Cellular hypertrophy	Amyloid degeneration (percent)	Nodular hyperplasia	Hepatocellular carcinoma
PCBs-5 (500)	+	+	++	0/12 —	7/12 (58.3)	5/12 (41.7)
(250)	±	±	+	2/12 (16.7)	0/12 —	0/12 —
(100)	−	−	±	3/12 (25.0)	0/12 —	0/12 —
PCBs-4 (500)	+	+	++	0/12 —	0/12 —	0/12 —
(250)	±	±	+	3/12 (25.0)	0/12 —	0/12 —
(100)	−	−	±	10/12 (83.3)	0/12 —	0/12 —
PCBs-3 (500)	−	−	±	1/12 (8.3)	0/12 —	0/12 —
(250)	−	−	±	4/12 (33.3)	0/12 —	0/12 —
(100)	−	−	−	10/12 (83.3)	0/12 —	0/12 —
Controls	−	−	−	0/6 —	0/6 —	0/6 —

*PCBs-5, Kanechlor 500; PCBs-4, Kanechlor 400; PCBs-3, Kanechlor 300.

Table D8 (from ref. 199)

—Histopathologic findings in the liver of male dd mice treated with isomers of benzene hexachloride (BHC) and technical polychlorinated biphenyls (PCBs) for 24 weeks

BHC or PCBs* in diet (ppm)	Effective number of mice†	Histopathology of liver			Liver nodules (percent)	
		Oval cells	Bile-duct prolif-eration	Cellular hypertrophy	Nodular hyperplasia	Hepato-cellular carcinoma
-BHC(250)	30	±	±	+++	23 (76.7)	8 (26.7)
α-BHC(250) + PCBs-5(250)	26	+	+	+++	21 (80.8)	15 (57.6)
α-BHC(100)	26	−	−	+	0 —	0 —
α-BHC(100) + PCBs-5(250)	25	±	±	++	8 (32.0)	1 (4.0)
α-BHC (50)	28	−	−	−	0 —	0 —
α-BHC (50) + PCBs-5(250)	30	−	−	++	9 (30.0)	2 (6.7)
β-BHC(250)	26	−	−	+	0 —	0 —
β-BHC(250) + PCBs-5(250)	29	±	±	+++	16 (55.2)	6 (20.7)
β-BHC(100)	26	−	−	−	0 —	0 —
β-BHC(100) + PCBs-5(250)	30	−	−	++	5 (16.7)	1 (3.3)
β-BHC (50)	28	−	−	−	0 —	0 —
β-BHC (50) + PCBs-5(250)	29	−	−	+	0 —	0 —
γ-BHC(250)	26	−	−	±	0 —	0 —
γ-BHC(250) + PCBs-5(250)	28	−	−	+	0 —	0 —
γ-BHC(100)	28	−	−	−	0 —	0 —
γ-BHC(100) + PCBs-5(250)	30	−	−	+	0 —	0 —
γ-BHC (50)	28	−	−	−	0 —	0 —
γ-BHC (50) + PCBs-5(250)	27	−	−	+	0 —	0 —
PCBs-5(250)	20	±	±	+	0 —	0 —
Controls	20	−	−	−	0 —	0 —

*PCBs-5, Kanechlor 500.

†Mice dying during the experiment were not included.

Histologically, the neoplasms induced in mouse liver by PCBs were nodular hyperplasia and well-differentiated hepatocellular carcinomas. They were similar to those described previously, produced on administration of the α-isomer of BHC (*14–16*), and to those induced by other chemical carcinogens (*17–25*).

Comment. Because of the very short periods of treatment and the small number of mice involved, especially in the control groups, this would be unacceptable as a routine carcinogenesis bioassay. No negative conclusions can be drawn from the failure of KC-400 or KC-300 to induce hyperplastic nodules or carcinomas within 32 weeks in these small groups of mice. However, their induction by KC-500 within 32 weeks is a striking positive finding. Survival of the mice was very good and the lesions were well illustrated.

The primary purpose of the experiment was to investigate synergistic effects of PCBs and BHC. The induction of hyperplastic nodules and carcinomas within 24 weeks by joint feeding of PCBs and BHC, at levels below those which had no effects in 32 weeks when fed independently, is a striking case of synergism. Because of the short duration of the experiment, this effect should probably be regarded as a case in which each chemical accelerated the action of the other. Lifetime studies at lower dietary levels would be required to demonstrate whether the life total incidence of carcinomas is increased by joint exposure.

D.8 Other Experiments on Co-Carcinogenesis in vivo.

Two other experiments have been conducted to investigate possible co-carcinogenic effects of PCBs in vivo. In an experiment with mice, PCBs (KC-400) fed in the diet did not appear to accelerate the development of cervical carcinoma induced by methylcholanthrene (272). After 4 weeks of topical application of methylcholanthrene, dysplastic changes in the cervix did not progress to invasive carcinoma in animals fed the control diet or PCBs, although they did so in animals fed DDT (272).

In another study in rats (273) feeding with PCBs (KC-500 at 500 ppm in the diet) for 24 weeks appeared to delay or inhibit the induction of nodular hyperplasia and hepatocellular carcinoma by three other carcinogens, 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB), N-2-fluorenylacetamide (2-FAA), and diethylnitrosamine (DEN). The results of this study are summarized in Table D9. The authors speculate that the inhibitory effects may have been attributable to metabolism of primary carcinogens by PCB-stimulated microsomal enzymes, as discussed in the next section.

D.9 Activation and De-activation of Primary and Secondary Carcinogens by PCB-Stimulated Microsomal Enzymes.

In a related experiment, Reynolds et al. (274) have shown that pretreatment with PCBs (150-300 µmoles/kg for 7 days) greatly increases the susceptibility of rat livers to acute injury by vinylchloride monomer (VCM). Twenty-four hours after a 6-hour exposure to

Table D9 (ref. 273)

Histopathologic changes of the liver in male rats treated with PCB and chemical carcinogens for 24 weeks

Group [a]	Effective No. of rats [b]	Histopathology of liver						
		Fatty changes	Cell hypertrophy	Oval cell infiltration	Bile duct proliferation	Cholangiofibrosis [c]	Nodular hyperplasia [c]	Cancer [c]
1. 3′-Me-DAB	23	+	±	+	+	1 (4.3)	22 (95.7)	15 (65.2)
2. 2-FAA	13	±	±	++	++	0	13 (100.0)	7 (53.8)
3. DEN	13	+	±	+	+	0	13 (100.0)	12 (92.3)
4. PCB	19	++	++	±	+	0	0	0
5. PCB+3′-Me-DAB	23	+	+	±	±	0	0	0
6. PCB+2-FAA	17	+	+	±	±	1 (5.9)	1 (5.9)	0
7. PCB+DEN	9	++	+	±	±	1 (11.1)	0	0
8. 3′-Me-DAB+DEN	13	+	±	++	++	4 (30.8)	13 (100.0)	12 (92.3)
9. 2-FAA+DEN	11	+	±	++	++	0	11 (100.0)	9 (81.8)
10. PCB+3′-Me-DAB+DEN	13	+	±	+	+	3 (23.1)	4 (30.8)	1 (7.7)
11. PCB+2-FAA+DEN	12	+	±	+	+	2 (14.3)	3 (25.0)	0
12. Control	10	−	−	−	−	0	0	0

[a] *See* footnote *a*, table 1.

[b] *See* footnote *c*, table 1.

[c] Number (and percent) of rats with cholangiofibrosis, nodular hyperplasia, or cancer of liver.

5% VCM, there was extensive vacuolization of the panlobular parenchyma in livers of PCB-treated rats, but not in livers of controls similarly exposed to VCM. VCM also caused extensive necrosis in PCB-treated rat livers, and a marked increase in serum alanine α-ketoglutarate transaminase (AKT) activity, a measure of general hepatic injury (Table D10). Phenobarbital (PBT) had similar effects (274, Table D10). Reynolds _et al_. related this injury to the oxidation of VCM to a biologically active metabolite by the mixed function oxidase system (MFOS) stimulated by PCBs and PBT. They showed that Aroclor 1254 and PBT have especially large effects in stimulating several components of the MFOS, especially cytochrome P-450, NADPH cytochrome c-reductase, and aryl hydrocarbon hydroxylase (AHH, as determined by zoxazolamine and 3,4-benzpyrene hydroxylase activities) (Figure D1). Correspondingly, Aroclor 1254 and PBT promote especially severe injury by VCM and large releases of AKT into the serum (Table D10); serum AKT is closely correlated with cytochrome P-450 content (Figure D2). By contrast the toxicity of 1,1-dichloroethylene (DCE, or vinylidene chloride) is sharply _reduced_ by pretreatment with PCBs and PBT (Table D10 and Figure D1), indicating that DCE is metabolized to a _less_ toxic compound. Figure D3 shows schematically how the MFOS acts to oxidize foreign chemicals, and demonstrates the key role of cytochrome P-450.

VCM is a known human liver carcinogen and its liver toxicity appears related to its carcinogenicity (275). Like most carcinogens, VCM is also a mutagen (276), but both its carcinogenicity and mutagenicity appear to depend on metabolism in the liver to an active

Table D10 (ref. 274)

Effect of MFOS Inducers on Serum Enzyme Response Following Vinyl Chloride and Dichloroethylene

Pretreatment	Serum AKT activity[*]	
	Vinyl chloride[†]	Dichloroethylene[‡]
Control (water)	0.32 ± 0.03	20.85 ± 3.96
PBT[§]	4.86 ± 2.69	11.85 ± 4.34
1254[‖]	5.55 ± 1.14	0.45 ± 0.01
HCB[§]	1.09 ± 0.30	26.02 ± 7.86
3-MC[§]	0.29 ± 0.06	13.94 ± 3.17
SNL[§]	0.28 ± 0.01	13.09 ± 1.47
PCN[§]	0.26 ± 0.01	11.19 ± 3.62

[*] Alanine ketoglutarate trasaminase activity (in milligrams of pyruvate per milliliter per hour) measured according to the method of Murphy and Malley[21]; the value from unexposed control animals was 0.38 ± 0.09 mg pyruvate/ml/hr.

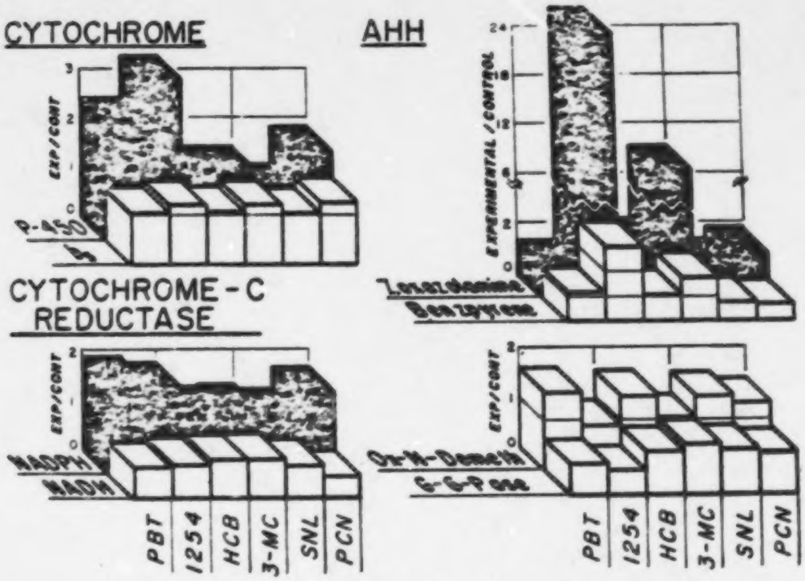
[†] Measured 24 hours after onset of a 6-hour exposure to 50,000 ppm.

[‡] Measured 6 hours after onset of a 4-hour exposure to 200 ppm.

[§] Seven-day pretreatment with 400 μmoles/kg/day.

[‖] Seven-day pretreatment with 300 μmoles/kg/day.

Figure D1 (ref. 274)



Comparison of the effects of the MFOS inducers, PBT, 1254, HCB, 3-MC, SNL, and PCN, on specific components of the microsomal multienzyme complex. All activities are on a milligram protein basis. The ordinate is expressed as the ratio of experimental to control values. Thus a value of one represents no change from control values. For the most part, each value is the average of the determinations from microsomes of at least 6 animals. Complete details of the assay conditions are described in Moslen *et al.*[31]

Figure D2 (ref. 274)

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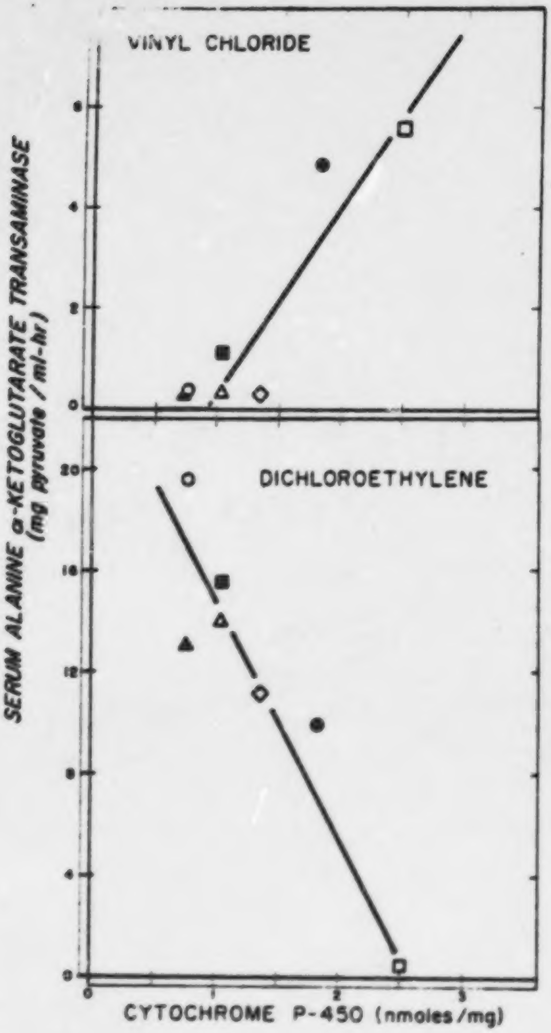
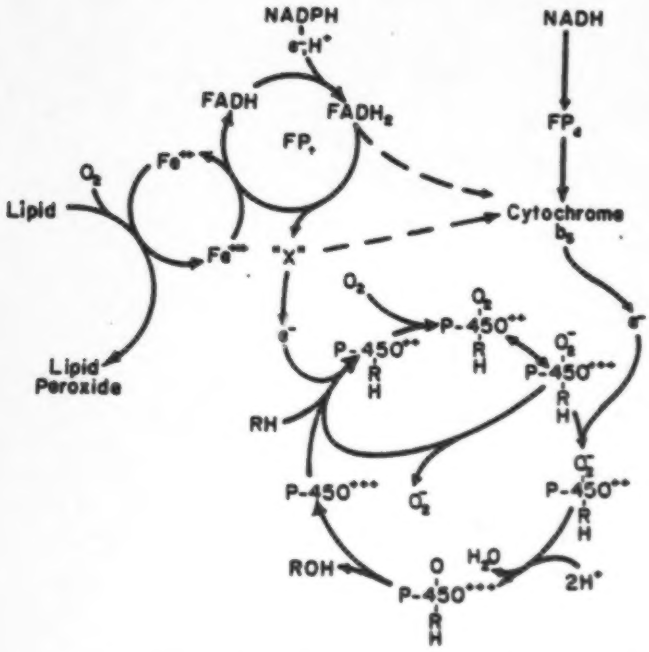


-Correlations between microsomal cytochrome P-450 content at the beginning of exposure and the serum enzyme response 24 hours after exposure to vinyl chloride and 6 hours after exposure to dichloroethylene in groups of animals given different MFOS inducers: controls, *open circles*; PBT, *solid circles*; 1254, *open squares*; HCB, *solid squares*; 3-MC, *open triangles*; SNL, *solid triangles*; PCN, *open diamonds*. Correlations between increased SAKT following VCM and cytochrome P-450 (r = 0.91) and between decreased SAKT following DCE and increased cytochrome P-450 (r = 0.95) are significant at the 1% level.

Figure D3 (ref. 274)



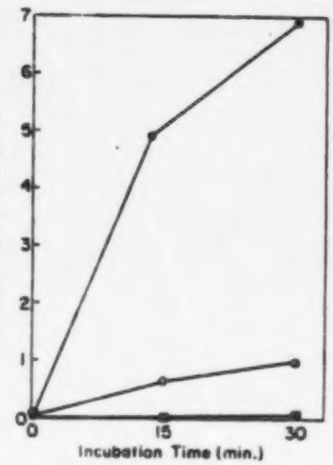
-Schematization of liver multimolecular mixed function oxidase (MFOS) system (adapted from: Estabrook, Concepts in Biochemical Pharmacology, 1971; Gillette *et al.*, Ann Rev Pharmacol 12:57, 1972; and Coon *et al.*, Drug Metab Disp 1:92, 1973) Electrons are transferred to the central hemoprotein (cytochrome P-450) through either the NADPH or NADH cytochrome P-450 reductase arms. For the monooxygenation process, Estabrook, Gillette, and colleagues have defined a sequence of reactions. First, the substrate (RH) combines with the oxidized form of cytochrome P-450. Second, this substrate–P-450 complex is reduced via a one electron transfer from the NADPH arm to form a reduced substrate P-450 complex. Third, the reduced complex then joins with molecular oxygen and in this triplet the oxygen is considered "active," possibly as a superoxide species with an electron resonating between the O_2 and the heme Fe. A second electron is then transferred in from either NADH or NADPH via cytochrome b_5, reducing the triplet, which decomposes to product, H_2O, and oxidized P-450. Coon *et al.*[29] have suggested additional possible roles for superoxide in hydroxylation reactions, including transfer of either electron or substrate attack with oxygen insertion.

metabolite, probably chloroethylene oxide (277). The mutagenicity of VCM has been demonstrated only in the presence of liver microsomal preparations, including those stimulated by PBT and PCBs (276-278).

This is only one of numerous instances in which PCBs have been shown to activate mutagens and carcinogens by stimulating the MFOS. It is known, for example, that polycyclic aromatic hydrocarbons (PAH) require metabolic activation via the formation of K-region epoxides and cis-dihydrodiols before they become mutagenically active in bacterial and mammalian cell lines (279). The AHH enzyme system responsible for the initial epoxidation of PAH is stimulated extremely efficiently by PCBs (274, Figure D1), and 18 different PAHs have been shown to be mutagenically active using PCB-stimulated MFOS-activation (276). Indeed, it has become standard practice to use Aroclor 1254 as a MFOS-stimulator in bacterial mutagenesis bioassays to screen suspected carcinogens (276, 280).

Chemical carcinogens may be classified as secondary or primary carcinogens according to whether or not they require metabolic activation before they become active carcinogens (and mutagens) (281). VCM, as stated above, appears to be a secondary carcinogen (277). Dimethylnitrosamine (DMN) also appears to be a secondary carcinogen and its activation to a mutagen appears to depend on oxidative demethylation by the cytochrome P-450 dependent MFOS (282). Both this process and the mutagenic activity of DMN in the presence of liver microsomes are enormously enhanced by exposure to single doses of Aroclor 1254 (281, 282) (Figure D4). By contrast N-methyl-N'-nitro-N-nitroso-guanidine

Figure D4 (ref. 282)



Frequency of DMN ilv^+ revertants of *B. subtilis* 168 *ilv*. The complete mixture contained 200 m*M* DMN, 10 m*M* $MgCl_2$, 50 m*M* DL-isocitrate-Na_3, 5 m*M* NADP, 0.2 mg isocitrate dehydrogenase, 15 mg microsomal protein and 3–7 × 10^8 bacterial cfu in 2.5 ml 0.1 *M* phosphate buffer at pH 7.4 and was incubated at 37°. In control mixtures (■—■) either microsomes or the NADPH generating system were omitted. The system with normal microsomes (○—○) contained 0.7 nmole P-450/mg microsomal protein, that with PCB induced microsomes (●—●) 2.5 nmole P-450/mg microsomal protein. The activity of aminopyrine demethylation expressed as nmole HCOH formed/mg protein/min was 10.2 and 25.4 for normal and PCB induced microsomes, respectively.

(MNNG) is a primary carcinogen and its mutagenic activity is reduced by pretreatment with PCBs (281).

These findings are of profound importance in evaluating the environmental significance of PCBs, because PCB mixtures, including both those with high and low percentages of chlorine (Aroclors 1260 and 1242/1016 respectively), have been found to stimulate MFOS at extremely low doses (219, 243). The significance of these findings has been enunciated by Popper *et al*. (281, pp. 728-729):

> The ability of isolated microsomes to modify the biological activity of mutagens confirms the suggestion that the microsomal biotransformation system plays a key role in chemical mutagenesis. Although there is probably a relationship between carcinogenesis and mutagenesis, there are still differences which have not been explained. The observation that induced microsomes inactivate the mutagenic activity of primary carcinogens or activate that of secondary ones to a greater extent than normal ones suggests that the status of microsomal metabolism affects the response of an individual to carcinogen exposure. If we are permitted to extrapolate from mutagenesis to carcinogenesis, we might understand some old observations. Differences in the status of the biotransformation system at the time of exposure may explain why only a limited percentage of exposed individuals develop cancer. Furthermore, nutritional disturbances, particularly protein (14) or riboflavin (15) deficiency, which reduce microsomal biotransformation, may explain the geographic prevalence of certain cancers such as those of the liver (16), because malnourished populations should be more susceptible to primary carcinogens. By contrast, in the well-nourished technologic societies, the biotransformation system induced by chemical pollutants, drugs and pesticides may prove to be the greater hazard.

APPENDIX E: METABOLISM AND BIODEGRADATION

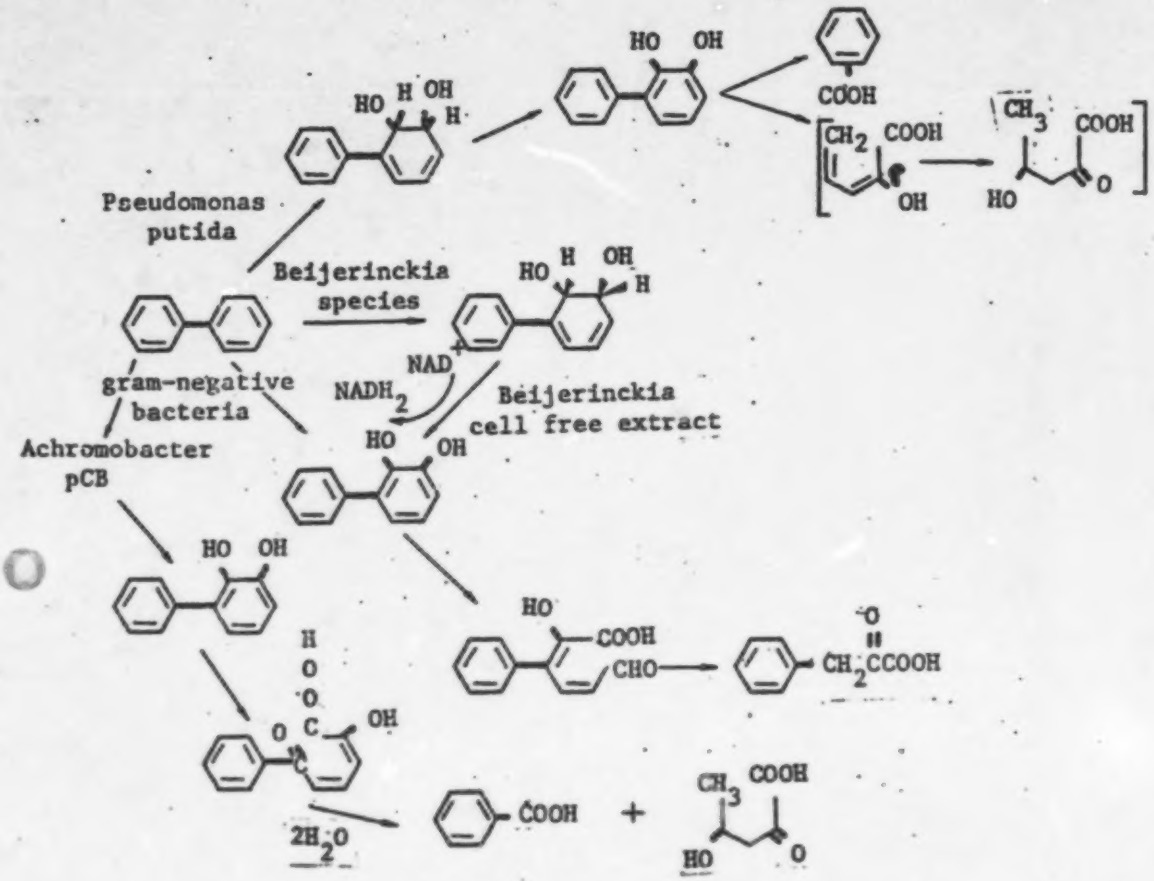
E.1. Metabolism by Bacteria

The ability of bacteria to degrade chlorinated biphenyls is dependent upon the degree of chlorination and chlorine substitution position as well as the stereo-chemistry and electronic structure of the chlorobiphenyl.

In laboratory investigations using single chlorobiphenyls and commercial mixtures, there is general agreement that lower chlorinated compounds can be degraded by microorganisms (see Figures E.2 and E.3; Tables E.1 and E.2), but there is little evidence for significant metabolism of tetra- and higher chlorobiphenyls. The mono- and dichlorinated biphenyls are readily degraded (82), as are chlorinated biphenyls with one unsubstituted ring (300).

The products resulting from microbial degradation of PCBs have been elucidated by several investigators. The products and intermediates obtained on incubating unchlorinated biphenyl with a number of microorganisms are shown in Figure E.1. If there are chlorines substituted, they appear to remain at their original point of attachment. For example, 4-chloro-biphenyl would degrade to 4-chlorobenzoic acid.

Ahmed and Focht (300) showed the preferential degradation of the unsubstituted ring of dichlorinated biphenyls using Achromobacter isolated from sewage effluent and grown on 4-chlorobiphenyl as the sole carbon source. Of the dichlorobiphenyls tested (2-3-; 2,4-;



Metabolic conversion of biphenyl by several microorganisms.
Figure E.1 (from refs. 4, 300, 301, 302)

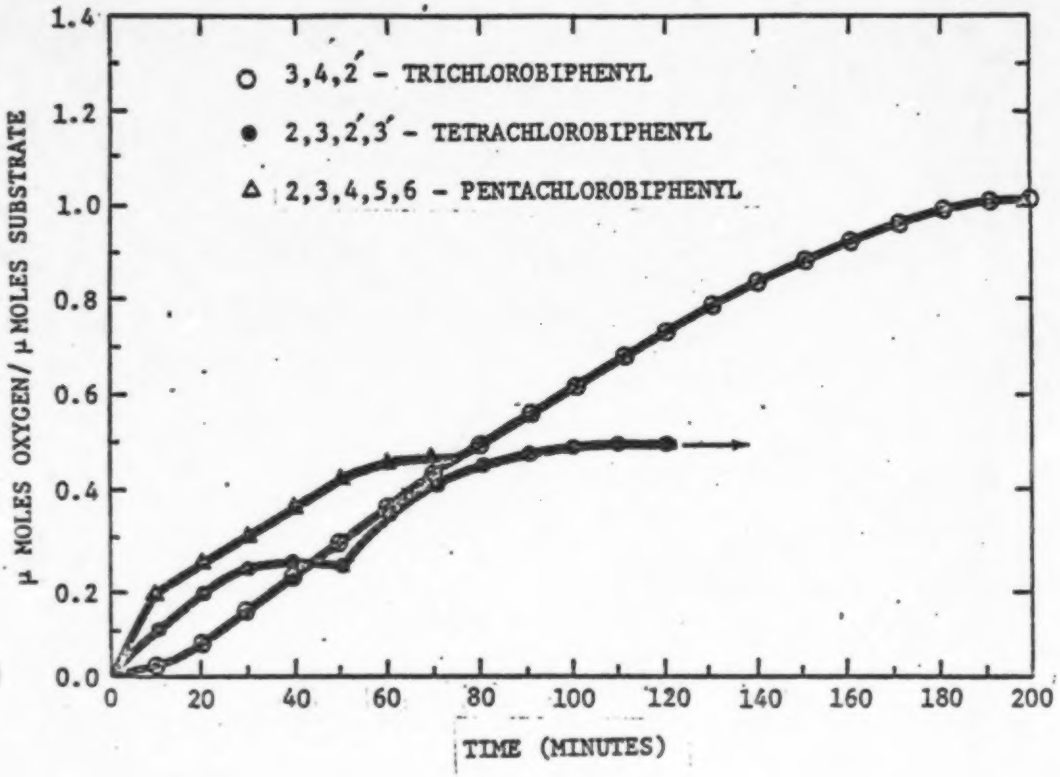
3,4-; 3,5-; and 3,3'-dichlorobiphenyl), only 3,3' DCB has both rings substituted. Figure E.2 shows the brief lag associated with oxidation of 3,3'-DCB while all other DCBs were oxidized without a lag. The tri-CB, tetra-CB and penta-CB were oxidized slightly with the penta-CB containing an unsubstituted ring oxidized more than the tetra-CB with two substituted rings (see Figure E.3). Manometric data were used to determine the extent of oxidation. Supernatant solutions indicated no chloride, further suggesting that chlorination renders the ring resistant to degradation and associated chlorine release.

Another experiment using bacteria (*Nocardia* spp. and *Pseudomonas* spp.) grown on biphenyl supports the proposition that low chlorinated biphenyls are readily degraded (82). The components of Aroclor 1242 tend to fall into three groups: (1) mono- or di-CBs which are degraded in less than 10 days; (2) tri-CBs which are degraded rapidly after an induction lag, then are degraded more slowly as their concentration drops to 10% of the original value; and (3) tetra-CBs which are degraded most slowly (see Figures E.4 and E.5). However, there were definite differences in degradation of the same compound alone or in combination (see Table E.1). For example, 4,4'-diCB, as a component of Aroclor 1242, was degraded almost completely in 20 days when exposed to *Nocardia* spp. (see Figure E.4), yet 4,4'-diCB showed no detectable change after 121 days when incubated alone with *Nocardia* spp. (see Table E.1). Mutual solubilization may be one mechanism involved, as supported by the degradation of hexaCB in combination with two triCBs and biphenyl, but not when the biphenyl was eliminated (see Table E.1). Co-metabolism has also been suggested to account for the microbial transformation of many pesticides in nature, compounds that do not supply the responsible populations with energy, carbon, nitrogen, or phosphorous, and may be another mechanism associated with the action of bacteria on PCBs. A more complete answer to the process of degradation is needed, as 4,4'-diCB could not be induced to degrade when mixed with 3,4,2'- and 2,3,2'-triCBs (82).

The evidence appears to support adaptation of the organisms. Thus, mono- and diCBs can probably be degraded with little further adaptation of the biphenyl-degrading bacteria (82). Increasing chlorination may require increasing induction time and be dependent upon the availability of precursors. The PCB degradation times probably reflect these adaptation periods (see Table E.1).

Wong and Kaiser (76) found that the ability of bacteria (Achromobacter sp. and Pseudomonas sp.), grown on 0.1% Aroclor 1242, to degrade the chlorinated biphenyl compounds in Aroclor 1242 mixture decreased with increasing chlorination. Specifically, the rates of degradation of biphenyl, 2-chlorobiphenyl and 4-chlorobiphenyl decreased in that order (see Figure E.6). Further, gas chromatography and mass spectrometry showed all metabolites to be aliphatic and aromatic compounds containing no chlorine. No phenols, alcohols or other oxidized derivatives of the PCBs were detected, indicating a rapid reduction of the expected intermediate hydroxylated metabolites to hydrocarbons.

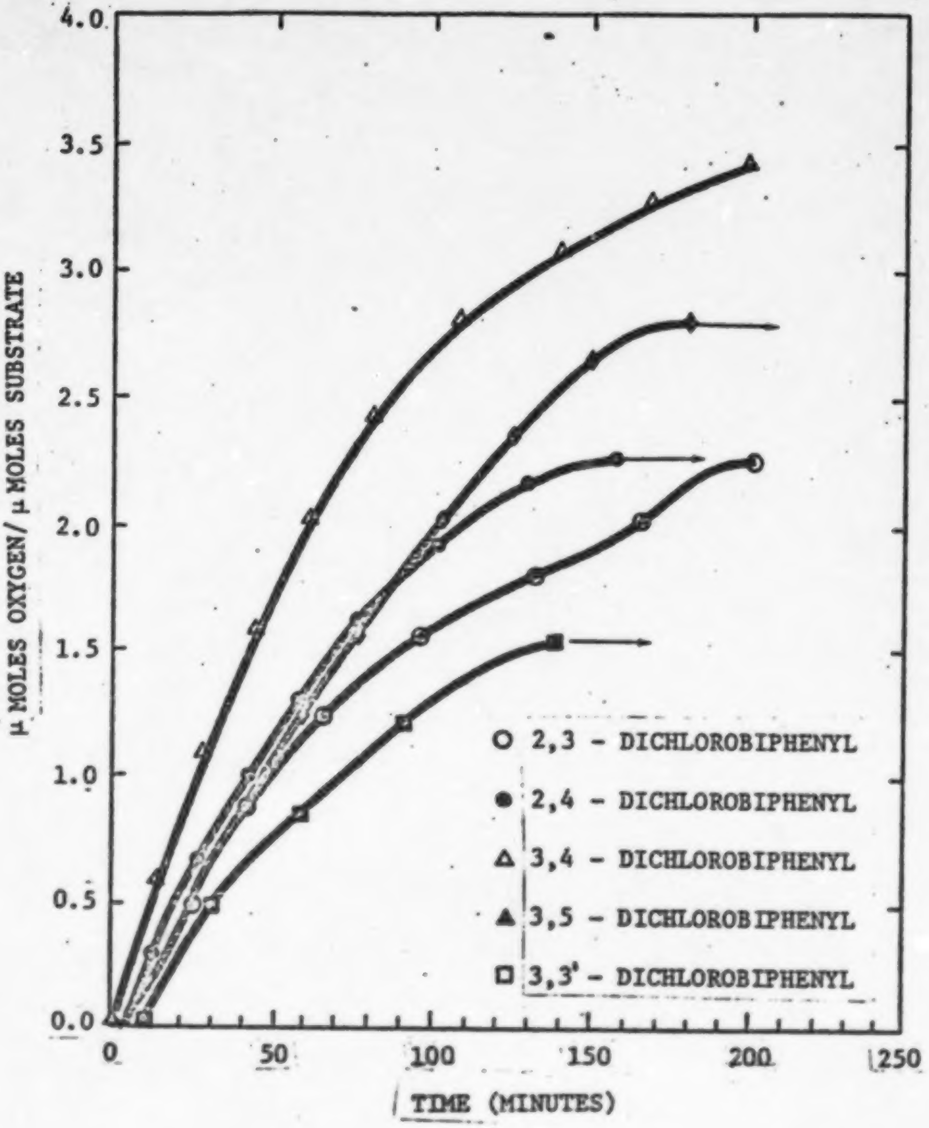
E 3a



Oxidation of tri, tetra, and penta chlorobiphenyls by Achromobacter pCB.

Figure E.3 (from ref. 300)

E 3b



Oxidation of dichlorobiphenyls by Achromobacter pCB.

Figure E.2 (from ref. 300)

Table E.1 PERCENTAGE LOSS OF PCBs AFTER A GIVEN PERIOD OF TIME
(from ref. 82)

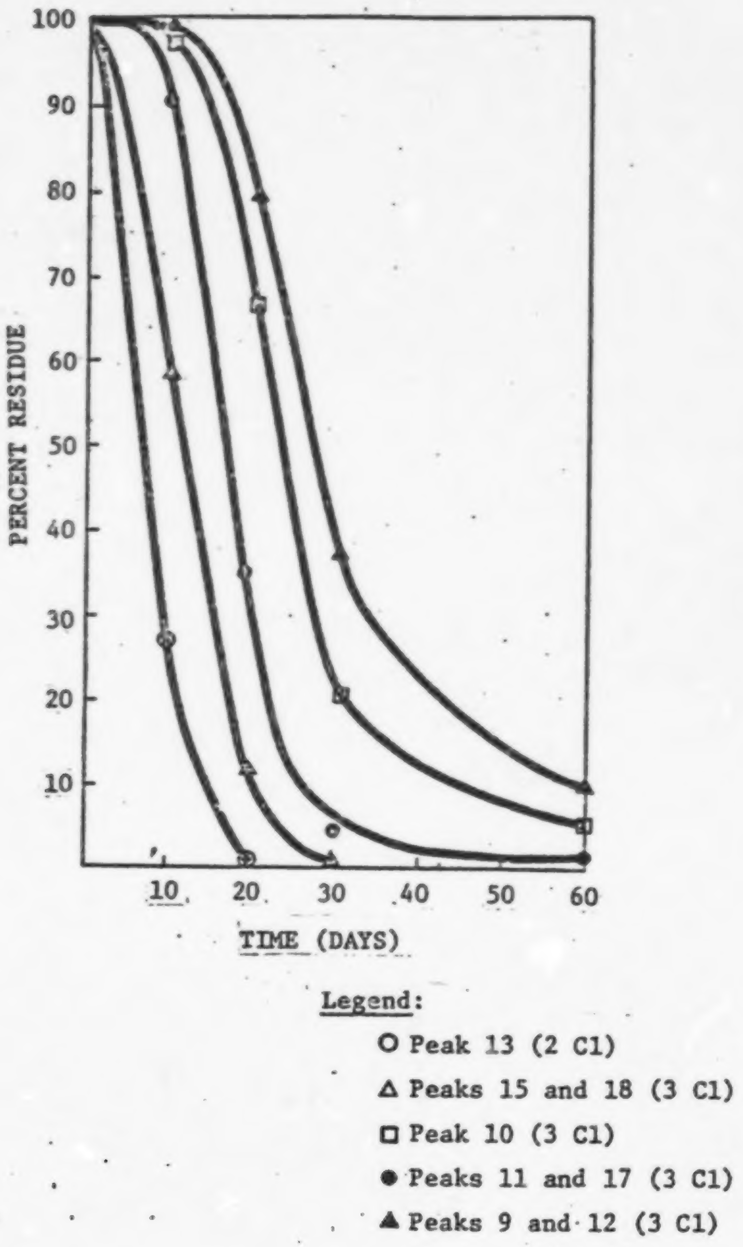
	% Degradation/Days	
Compound	*Nocardia* spp.	*Pseudomonas* spp.
2,4'-Dichlorobiphenyl	70/7	60/73
2,4'-Dichlorobiphenyl + biphenyl	100/7	100/73
4,4'-Dichlorobiphenyl	nil/121	50/15
4,4'-Dichlorobiphenyl + biphenyl	nil/121	50/10
2,3-Dichlorobiphenyl	67/8	--
2,3-Dichlorobiphenyl + biphenyl	64/8	--
3,4-Dichlorobiphenyl	80/8	--
3,4-Dichlorobiphenyl + biphenyl	100/8	--
2,3,2'-Trichlorobiphenyl	50/7	--
2,3,2'-Trichlorobiphenyl + biphenyl	95/7	--
2,3,4'-Trichlorobiphenyl	94/7	--
2,3,4'-Trichlorobiphenyl + biphenyl	100/7	--
2,5,4'-Trichlorobiphenyl	nil/73	15/73
2,5,4'-Trichlorobiphenyl + biphenyl	60/73	60/73
3,4,3'-Trichlorobiphenyl	76/12	--
3,4,3'-Trichlorobiphenyl + biphenyl	70/12	--
2,4,6-Trichlorobiphenyl	nil/12	nil/84
2,4,6-Trichlorobiphenyl + biphenyl	nil/12	nil/84
2,4,2',4'-Tetrachlorobiphenyl	nil/9	--
2,4,2',4'-Tetrachlorobiphenyl + biphenyl	nil/9	--
2,4,6,2'-Tetrachlorobiphenyl	nil/9	--
2,4,6,2'-Tetrachlorobiphenyl + biphenyl	nil/9	--
2,3,4,5,2',3'-Hexachlorobiphenyl + 2,3,2'- and 2,3',4'-trichlorobiphenyl	nil	--
2,3,4,5,2',3'-Hexachlorobiphenyl + 2,3,2'- and 2,3',4'-trichlorobiphenyl + biphenyl	50/11	--

E 3d

Table E.2
(from ref. 82)

PERCENTAGE DEGRADATION

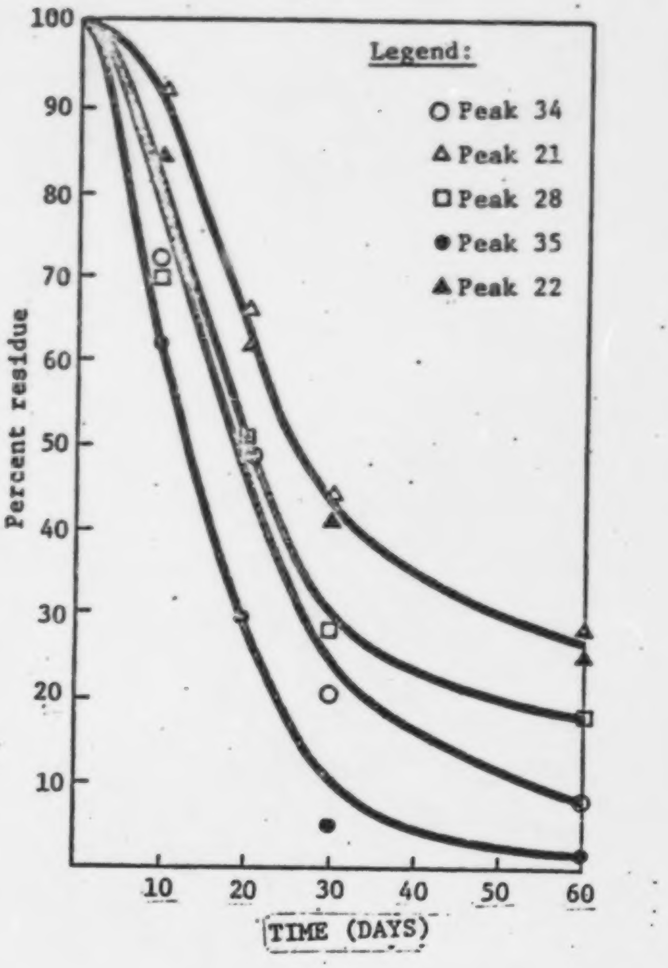
	Aroclor 1242		Aroclor 1016	
Time (days)	*Nocardia* spp.	*Pseudomonas* spp.	*Nocardia* spp.	*Pseudomonas* spp.
52	88	76	96	91
100	95	85	>98	>96



Degradation of typical di- and tri-chlorobiphenyl components of Aroclor 1242 following exposure to Nocardia spp.

Figure E.4 (from ref. 82)

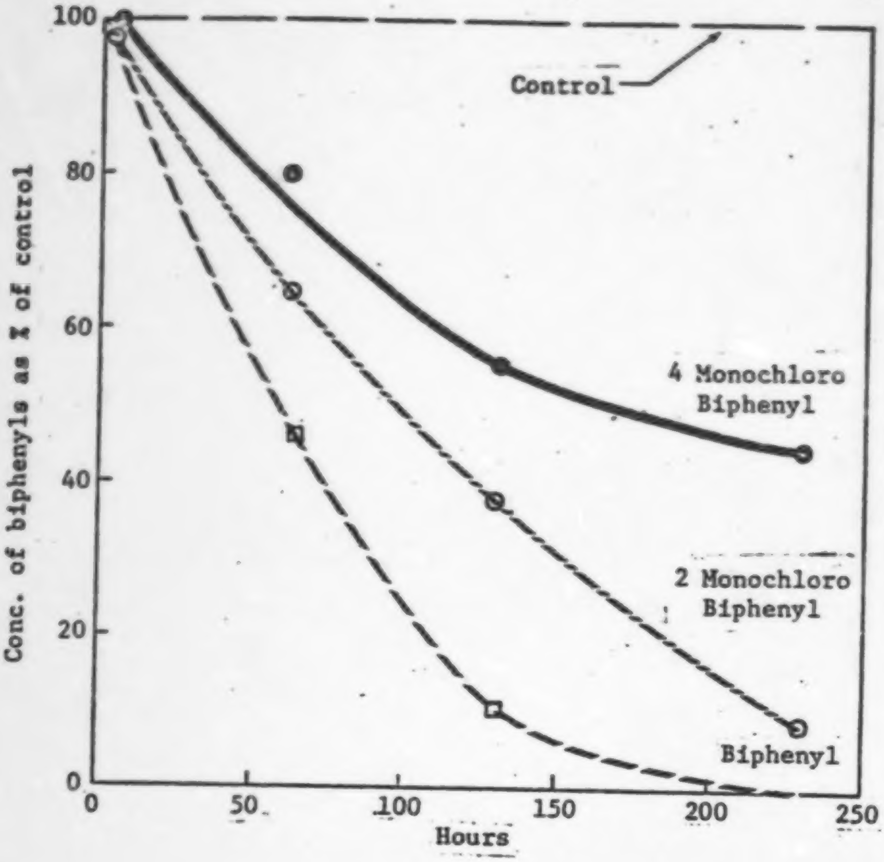
E 3f



Degradation of typical tetra-chlorobiphenyl components of Aroclor 1242 following exposure to Nocardia spp.

Figure E.5 (from ref. 82)

E 3g



Concentrations of biphenyl, 2-chlorobiphenyl and 4-chlorobiphenyl in the growth medium after bacterial degradation during three incubation periods.

Figure E.6 (from ref. 76)

The slower degradation of more highly chlorinated PCBs is illustrated by the observation of Veight as reported in Wong and Kaiser (76) that Aroclor 1242 concentration decreased at a faster rate than Aroclor 1260 in lake water, but no data were given. Tucker _et al_. (303) found an inverse relationship between degradation rate and chlorine content in commercial PCBs (see Figure E.7) using supernatant samples from a semi-continuous activated sludge technique*.

Effluent from a secondary sewage treatment laboratory system contained relatively small amounts of the seeded Aroclor 1254, with a considerable PCB accumulation in the sludge (304). By sampling only the supernatant, Tucker _et al_. (303) may have mistaken PCB removal to sludge through fat dissolution, adsorption on particle surfaces, and microbial ingestion, for degradation.

Other investigations using naturally occurring microorganisms have produced results indicating no metabolism of chlorinated biphenyls. Fries, as reported in Hutzinger _et al_. (4), found no preferential degradation of Aroclor 1254 components in silage after several months of normal fermentation.

In an experiment using 0.1 ppm Aroclor 1260 added to natural river and marine waters, Oloffs _et al_. (41) could detect no metabolites;

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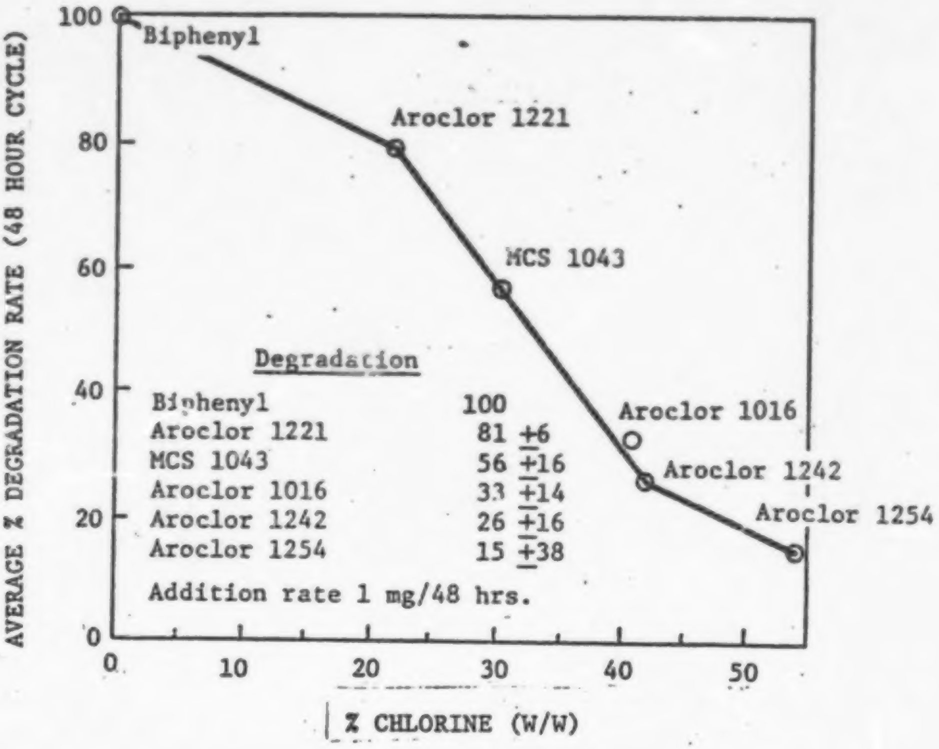
* This procedure consisted of mixing activated sludge cultures from a sewage treatment plant with synthetic sewage and allowed to acclimate for several weeks before adding polychlorinated biphenyl mixtures. Aeration was interrupted for sampling and at the end of a cycle when the sludge was allowed to settle before withdrawing supernatant. After replacement of the fluid with tap water, the cycle was reinitiated by adding synthetic sewage and PCBs.



Semi-continuous activated sludge primary biodegradation rates of commercial PCBs as a function of the weight percent chloride.

Figure E.7 (from ref. 303)

but parent compounds were recovered in decreased concentration. Two mechanisms were postulated for Aroclor 1260 loss from water: codistillation and evaporation. Although 0.1 ppm Aroclor 1260 evinced a slight enhancement of bacterial growth, metabolic breakdown probably would be very slow at the natural temperatures maintained (7 and 16°C), one possible explanation for the lack of detectable metabolites.

Cultures isolated on biphenyl media can provide both induced bacteria and greater numbers of the adapted organisms. Wong and Kaiser (76) found that only 1% of the bacteria from nutrient agar could grow on 0.01% Aroclor 1221 enriched agar. Baxter et al. (82) suggested that degradation patterns of Aroclor 1242 could be explained in terms of adaptation of Nocardia sp. grown on biphenyl. Thus, little further adaptation was necessary to degrade mono- and diCBs, but for more highly chlorinated components, increasing adaptation may be required (see Figures E.4 and E.5).

Soil microorganism metabolism of PCBs appears to depend on soil type (4, 305). No indication of any metabolism was found in soil alone or soil enriched with cattle manure in which single chlorobiphenyls (4,4'-di; 2,5,2',5'-, 2,6,2',6'-, 3,4,3',4'-, and 3,5,3',5'-tetra; 2,4,5,2',5'-penta; and 2,4,5,2',4',5'-, and 2,4,6,2',4',6'-hexaCB) were incubated under flooded conditions in the laboratory for one month (306). Under the same conditions, p,p'-DDT degrades almost completely; therefore, the degradation pathways for these compounds are not the same.

E.2. Metabolism by Invertebrates

Metcalf *et al*. (45) studied the metabolism of three representative chlorobiphenyls in a model ecosystem which included several invertebrate animals. Their findings in the salt-marsh caterpillar (*Estigmene acrea*) are shown in Table E.3. Although 76% of the tri-CB was metabolized and excreted as polar degradation products, only 4.6% and 2.1% of the tetra- and penta-CBs were so degraded. Similar results were obtained with a mosquito larva (*Culex pipiens*) and a snail (*Physa*) (Table E.4). Small amounts of non-polar metabolites were found also, especially in the case of the tri-CB: much of this non-polar material was retained in the tissues of the organisms and not excreted (Tables E.3 and E.4).

E.3. Metabolism by Fish

Table E.4 also gives the results of Metcalf *et al*.'s study of metabolism of the same three representative chlorobiphenyls in mosquito fish (*Gambusia*). As in the case of the invertebrates, a substantial fraction of the tri-CB was metabolized, but only insignificant fractions of the tetra- and penta-CBs. Metcalf *et al*. (45) define a "biodegradability index": the ratio of polar to non-polar metabolites. This index ranged from 0.17 to 0.60 for the tri-CB, indicating substantial metabolism to polar products, but was less than 0.082 for all organisms for the tetra- and penta-CBs, indicating negligible biodegradation (Table E.5).

E 6a

Table E.3
(from ref. 45) *Metabolism of ^{14}C radiolabeled compounds by salt marsh caterpillar*[a]

	Body	Feces
A. 2,5,2′-trichlorobiphenyl total ^{14}C (%)	8.66	91.34
Unknown I (R_f 0.53[a])	0.64	–
trichlorobiphenyl (R_f 0.43)	5.84	8.91
Unknown II (R_f 0.31)	0.27	0.37
Unknown III (R_f 0.13)	0.05	0.12
Unknown IV (R_f 0.05)	0.10	4.67
Unknown V (R_f 0.02)	0.11	0.92
Polar (R_f 0.0)	1.65	76.35
B. 2,5,2′,5′-tetrachlorobiphenyl total ^{14}C (%)	78.68	21.32
tetrachlorobiphenyl (R_f 0.50[a])	75.60	15.08
Unknown I (R_f 0.41)	0.99	1.36
Unknown II (R_f 0.05)	0.13	–
Unknown III (R_f 0.03)	trace	0.20
Polar (R_f 0.0)	1.96	4.64
C. 2,5,2′,4′,5′-pentachlorobiphenyl total ^{14}C (%)	75.86	24.14
pentachlorobiphenyl (R_f 0.53[a])	74.00	20.70
Unknown I (R_f 0.46)	0.74	0.74
Unknown II (R_f 0.39)	0.62	0.56
Unknown III (R_f 0.03)	0.08	0.08
Polar (R_f 0.0)	0.42	2.06
D. 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE) total ^{14}C (%)	80.59	19.41
DDE (R_f 0.49[a])	76.88	19.37
Polar (R_f 0.0)	3.71	0.04

[a] TLC with hexane (Skellysolve B, bp 60-68°C).

Table E.4
(ref. 45)

Distribution of chlorinated biphenyls and their degradation products in the model ecosystem

	Chlorinated biphenyl equivalents (ppm)				
	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
I. 2,5,2′-trichlorobiphenyl total ^{14}C	0.03845	23.2155	31.2015	2.7030	3.2055
Unknown I (R_f 0.66^{a})	0.00015	15.9575	18.9720	1.1995	0.2085
trichlorobiphenyl (R_f 0.56)	0.00020	1.4630	1.1590	0.1630	1.2800
Unknown II (R_f 0.23)	0.00005	0.0520	0.6480	+	0.1595
Unknown III (R_f 0.10)	–	–	0.9735	–	–
Unknown IV (R_f 0.06)	0.00055	–	0.5460	–	–
Unknown V (R_f 0.04)	0.00040	–	0.2205	–	–
Unknown VI (R_f 0.03)	0.00040	0.0685	0.4410	–	–
Polar (R_f 0.0)	0.02265	0.5185	3.9315	0.4795	0.9985
Unextractable	0.01405	5.1560	4.3100	0.8610	0.5590
II. 2,5,2′,5′-tetrachloro biphenyl total ^{14}C	0.02065	23.6845	53.7465	14.5335	15.5685
tetrachlorobiphenyl (R_f 0.48^{a})	0.00120	21.5975	47.3275	12.6745	14.2360
Unknown I (R_f 0.23)	0.00005	0.3220	0.7560	0.1070	0.0890
Unknown II (R_f 0.04)	0.00155	0.1030	0.4360	–	–
Polar (R_f 0.0)	0.01225	0.3275	3.9850	0.9670	0.8545
Unextractable	0.00560	1.3345	1.2420	0.7850	0.3900
III. 2,5,2′,4′,5′-pentachlorobiphenyl total ^{14}C	0.04340	62.4660	633.0165	181.4565	127.6945
pentachlorobiphenyl (R_f 0.55^{a})	0.00985	53.8440	587.3545	170.8480	119.7060
Unknown I (R_f 0.46)	–	0.6850	8.6210	2.4070	2.5380
Unknown II (R_f 0.39)	0.00020	0.5080	2.2490	1.3195	0.5810
Unknown III (R_f 0.21)	0.00015	0.1425	1.9365	1.0520	0.3285
Unknown IV (R_f 0.04)	0.00030	–	0.5000	–	–
Unknown V (R_f 0.02)	0.00385	0.2570	7.4965	–	0.7450
Polar (R_f 0.0)	0.02055	1.6265	16.5550	2.6745	2.3610
Unextractable	0.00850	5.4330	8.3040	3.1555	1.4350

[a] TLC with hexane (Skellysolve B,bp 60-68°C).

E 6c

Table E.5
(ref. 45)

Ecological magnification (E.M.) and Biodegradability index (B.I.) of PCB's and DDE compared with water solubility and partition coefficient

Chemical	H_2O solubility (ppb)	Partition coefficient	Ecological magnification (E.M.)				Biodegradability index (B.I.)			
			Alga	Snail	Mosquito	Fish	Alga	Snail	Mosquito	Fish
tri-Cl-PCB	16	7,803	7,315	5,795	815	6,400	0.30	0.17	0.35	0.60
tetra-Cl-PCB	16	8,126	17,997	39,439	10,562	11,863	0.015	0.082	0.076	0.060
penta-Cl-PCB	19	16,037	5,464	59,629	17,345	12,152	0.029	0.027	0.0134	0.019
DDE	1.3	18,893	11,251	36,342	59,390	12,037	0.069	0.049	0.033	0.050

Sanborn et al. (307) obtained similar results with the green sunfish (Lepomis cyanellus) which was able to metabolise about 80% of the tri-CB, but almost none of the tetra- or penta-CB.

By contrast, Hutzinger et al. (308) were unable to detect any metabolites formed by brook trout dosed with representative mono-, di-, tetra- and penta-CBs. There was little, if any, metabolism of a penta-CB by the dogfish (309).

E.4. Metabolism by Birds

Hutzinger et al. (308) reported that pigeons were able to metabolize mono-, di- and tetra-CBs, but not a hexa-CB. The metabolites identified were mono-hydroxy -CBs; unlike rats, pigeons did not produce detectable quantities of di-hydroxy-CBs. De Freitas and Norstrom (27) reported a relation between ease of "metabolism" in pigeons and chlorine substitution patterns, but their measurements were based on differential retention and did not in fact distinguish between metabolism and excretion.

McKinney et al. (29, 310) have recently studied the metabolism of five symmetrical isomers of hexa-CB in chickens, with some novel and important results. In addition to the hydroxylated metabolites identified by others as the major metabolites in birds and mammals, they found evidence of isomerization (rearrangement of chlorine substitutions), dechlorination, and formation of CDFs. The metabolites of 2,4,5,2',4',5'-hexaCB included a penta-CB, a mono-hydroxy derivative, together with the 3,6-quinol and 3,6-quinone derivatives of the hexa-CB. The metabolites of 2,4,6,2',4',6'-hexaCB included a rearrangement product (2,4,6,2',3',4'-hexaCB), a penta-CB, a hexa-CDF and a penta-CDF. The other three hexaCBs studied (2,3,6,2',3',6'-; 2,3,4,2',3',4'-; and 3,4,5,3',4',5'-) showed little or no metabolism except in the first case, in which a mono-hydroxy derivative was formed. The formation of the two CDFs by metabolism of chlorobiphenyls appears to be well established by gas chromatography--mass spectrometric techniques (310).

The hexa-CDF was the most abundant chlorinated compound present in the feces of the chicks, indicating that it was formed in significant quantities. The authors (210) suggest that it is formed via ortho-hydroxylation in association with chlorine migration, and hence suggest that it may have a 1,2,3,7,8,9 substitution pattern. This newly-discovered phenomenon is of potentially great toxicological significance.

E.5. Metabolism in Mammals

Metabolism of chlorobiphenyls has been extensively studied in mammals. A concise summary of recent work on metabolism in the rat has been published by Goto et al. (311: Figure E.9); as shown in this section, metabolism in the other mammalian species studied to date appears to be generally similar.

The liver appears to be the main site of metabolism of PCBs. The hypothesis that PCBs are metabolically degraded by the liver is supported by studies showing that the biotransformation of PCBs is markedly retarded when livers are damaged by administration of carbon tetrachloride. Grant et al. (182) found that residues of Aroclor 1254 in rat tissues were higher in rats simultaneously treated with carbon tetrachloride than in rats dosed only with Aroclor 1254. The residue pattern in fat of animals treated with carbon tetrachloride was similar to that of standard Aroclor 1254, indicating little metabolism.

Hydroxylation appears to be the main metabolic route for chlorobiphenyls (4, 28, 308-314). Generally, dechlorination does not seem to take place. Hutzinger et al. (4) did find traces of substances in rat feces whose mass spectra indicated loss of a chlorine atom. The spectra indicated a trichlorinated metabolite of 3,4,3',4'-tetraCB and a heptachlorinated metabolite of 2,3,4,5,2',3',4',5'-octaCB. Hutzinger et al. have also identified a dechlorinated metabolite of 2,4,5,2',4',5'-hexaCB in rabbits (315).

Hutzinger et al.(4) studied metabolic behavior of 11 chlorobiphenyls in rats after intraperitoneal injection. Extracts of urine and feces were analyzed by thin layer chromatography and mass spectrometry. The identities of many metabolites were confirmed by NMR spectroscopy and synthesis. The results of this study are summarized in Table E.6. They did not find metabolites for hexa and octachlorobiphenyls. Decachlorobiphenyl was not soluble enough in oil for injection into rats.

Work has been done on metabolism of individual chlorobiphenyls.

E.9a

POLAR METABOLITES OF CHLOROBIPHENYLS FOUND IN DIFFERENT ANIMAL SPECIES

Table E.6 (from ref. 4)

Chlorobiphenyl Administered				Metabolite Found		
				Monohydroxy* Compound	Dihydroxy* Compound	
No.	Name	Formula	Animal Species Used	M* = m/e Chlorobiphenyl+16	M* = m/e Chlorobiphenyl+32	Other Derivative
I	4-Chlorobiphenyl		rat	+	+	-
			pigeon	+	-	-
			trout	-	-	-
			rabbit	+	-	-
II	4,4'-Dichlorobiphenyl		rat	+	+**	-
			pigeon	+	-	-
			trout	-	-	-
III	2,2'-Dichlorobiphenyl		rat	+		-
IV	2,4'-Dichlorobiphenyl		rat	+	-	-
V	2,2',4,4'-Tetrachlorobiphenyl		rat	+	-	-
VI	2,2',5,5'-Tetrachlorobiphenyl		rat	+	-	-
			pigeon	+	-	-
			trout	-	-	-
			rabbit	+	-	Dihydrodihydroxy-tetrachlorobiphenyl
VII	3,3',4,4'-Tetrachlorobiphenyl		rat	-	-	-
VIII	2,2',4,4',5,5'-Hexachlorobiphenyl		rat	-	-	-
			pigeon	-	-	-
			trout	-	-	-
			rabbit	+	-	Hydroxypentachlorobiphenyl hydroxymethoxypentachlorobiphenyl
IX	2,2',4,4',6,6'-Hexachlorobiphenyl		rat	-	-	-
X	2,2',3,3',5,5'-Hexachlorobiphenyl		rat	-	-	-
XI	2,2',3,3',4,4',5,5'-Octachlorobiphenyl		rat	-	-	-

* Including conjugates.
** Detected by dansylation procedure.

Monochlorobiphenyls. Hutzinger et al. (308) found that 4-chlorobiphenyl in rat urine metabolized to mono- and dihydroxychlorobiphenyl. Very little starting material could be recovered from the feces. No starting material could be recovered from the urine. These observations indicate extensive metabolic degradation.

Safe et al. (316) in studies of male Wistar rats, employed NMR spectroscopy to establish the hydroxylation of 4-chlorobiphenyl at the 4' position.

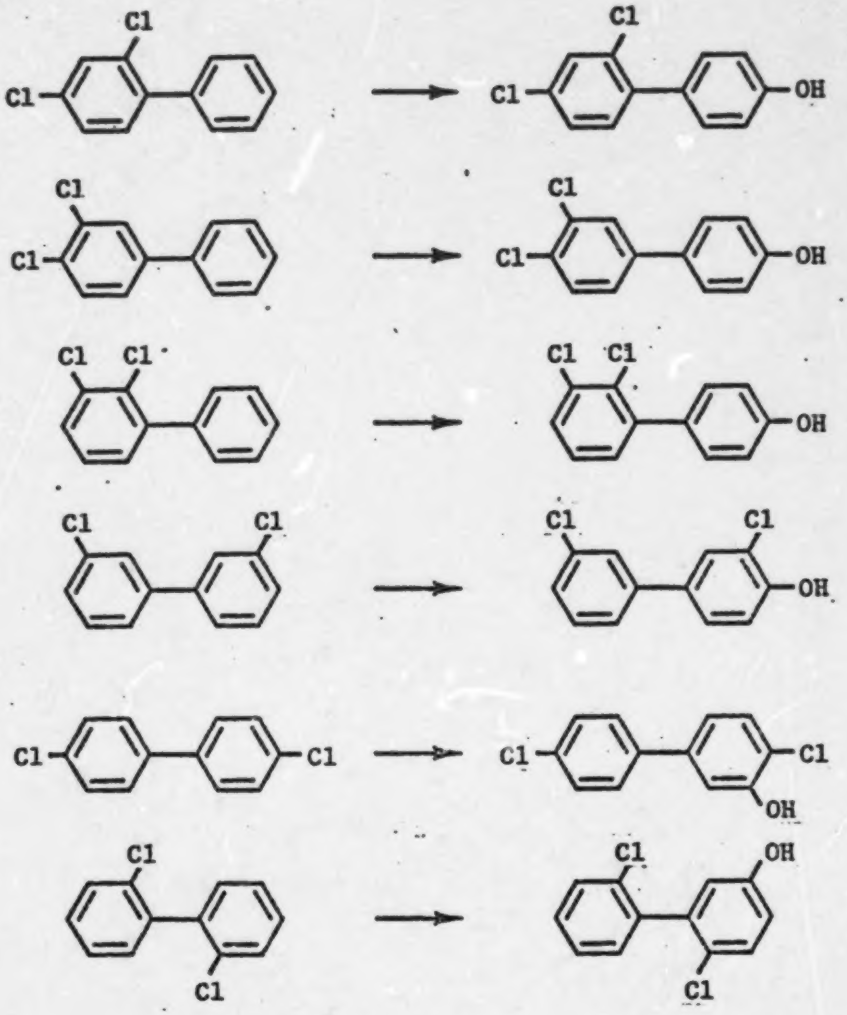
Di- and Trichlorobiphenyls. Greb et al. (317) found that mono and dihydroxylated derivatives were the major urinary metabolites of 2,4'-dichlorobiphenyl and 2,2',5-trichlorobiphenyl in the rhesus monkey. They found similar metabolites of these two compounds to be produced by rat liver microsomes (318).

Hutzinger et al. (308) reported that large quantities of unchanged 4,4'-dichlorobiphenyl could be extracted from rat feces, but only its monohydroxy metabolites could be found in rat urine.

Hutzinger et al. (4) present a number of monohydroxy derivatives observed as metabolic products from specific CBs in rats (Figure E.8). They also observed dihydroxy metabolites of 2,2'- and 4,4'-diCBs.

Safe et al. (316) used NMR spectroscopy to elucidate the structure of the major hydroxylated metabolite of 4,4'-dichlorophenyl fed to rats. They found that hydroxylation occurred at the 3 position to produce 4,4'-dichloro-3-hydroxybiphenyl. A similar result was obtained in goats and cows (324).

E 10a



Monohydroxy-derivatives observed as metabolic products from dichlorobiphenyls in rats.

Figure E.8 (from ref. 4)

Tetrachlorobiphenyls. Yoshimura and Yamamoto (313) studied the metabolic fate of 3,4,3',4'-tetrachlorobiphenyl in rats. They found 64% of the dose excreted unchanged in the feces. They felt that most of this unchanged material had not been absorbed from the gastrointestinal tract. At least three metabolites were also excreted in the feces. The major metabolite, a mono-hydroxy derivative, excreted during 14 days accounted for only about 3.3% of the dose administered.

The metabolic fate of 2,4,3',4'-tetraCB was also investigated. At least four metabolites of phenolic nature were excreted exclusively into the feces along with a large amount of 2,4,3',4'-tetraCB. The major metabolite was identified as 5-hydroxy-2,4,3',4'-tetraCB (312).

Yoshimura et al. (314) found that the metabolic rate for 2,4,3',4'-tetraCB seemed different from that of 3,4,3',4'-tetraCB despite the fact that all tetraCB metabolites they found in rat feces were monohydroxylated derivatives.

Gardner et al. (319) used infrared and mass spectra to identify the urinary metabolites of 2,5,2',5'-tetraCB in rabbits. Three hydroxylated metabolites were excreted. They were:

3-hydroxy-2,5,2',5'-tetraCB
4-hydroxy-2,5,2',5'-tetraCB
trans-3,4-dihydro-3,4-dihydroxy-2,5,2',5'-tetraCB

The first two compounds are phenols, but the third is a dihydrodiol with the vicinal hydroxy groups in the trans conformation.

Van Miller _et_ _al_. (320) studied the metabolic fate of the same tetra-CB in rats and found that it was rapidly metabolized to one or more hydroxylated metabolites and excreted. The principal metabolite identified was 3-hydroxy-2,5,2',5'-tetraCB. Little of the material was stored in the fat.

Hsu _et_ _al_. (321) found distinct differences in the metabolism of the same tetra-CB in infant rhesus monkeys. The 3-hydroxy derivative was not found: one of two metabolites was unidentified; the other was identified as the trans-3,4-dihydroxy derivative. Hsu _et_ _al_. suggest that the mechanisms of metabolism in rats and monkeys must differ (see below).

Pentachlorobiphenyls. Berlin _et_ _al_. (322) treated mice with 2,4,5,2',5'-pentaCB and found that 80% was excreted within 19 days, mostly in the form of a mono-hydroxy metabolite.

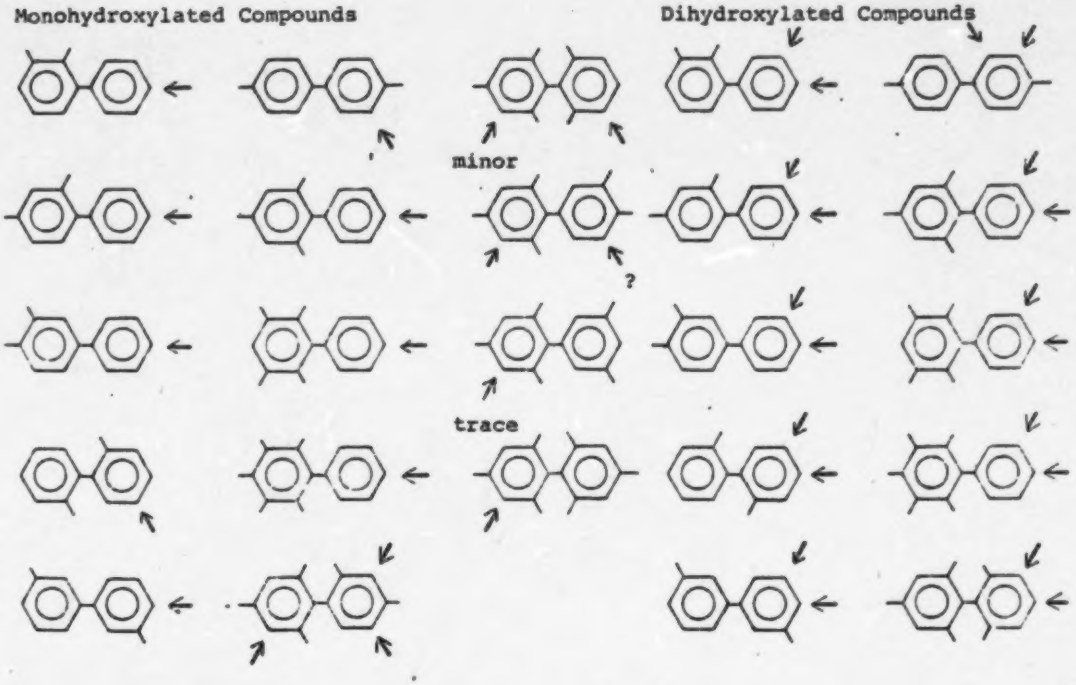
Hexachlorobiphenyls. Metabolic dechlorination of 2,4,5,2',4',5'-hexachlorobiphenyl was found by Hutzinger _et_ _al_. (315). Rabbits were fed a diet containing 1% 2,4,5,2',5',5'-hexachlorobiphenyl for seven days. The total concentration of chlorobiphenyl administered was 1 g per kg. Urine, collected during the entire feeding period, was collected, extracted and analyzed by a high resolution mass spectrometric method involving photoplate detection. Two major metabolites and a minor metabolite were detected. The first major metabolite was formed from the hexachlorobiphenyl by hydroxylation and the second by hydroxylation with concomitant dechlorination. Formation of the minor metabolites also involved dechlorination.

Jensen and Sundström (323) also found a mono-hydroxy derivative of this hexa-CB, probably the 3-hydroxy derivative, in the feces of rats. This is an important finding because 2,4,5,2',5',5'-hexaCB has no adjacent unsubstituted carbon atoms, so that its hydroxylation must take place directly (323).

Summary of metabolism in the rat. Goto et al. (311) have summarized their own work and that of others on the metabolism of chlorobiphenyls (as shown in Figure E.9). These results show that hydroxylation usually takes place at the 3- and 4- positions, rarely at the 2-position. With a few exceptions, hydroxylation usually occurs at one of two adjacent unsubstituted positions.

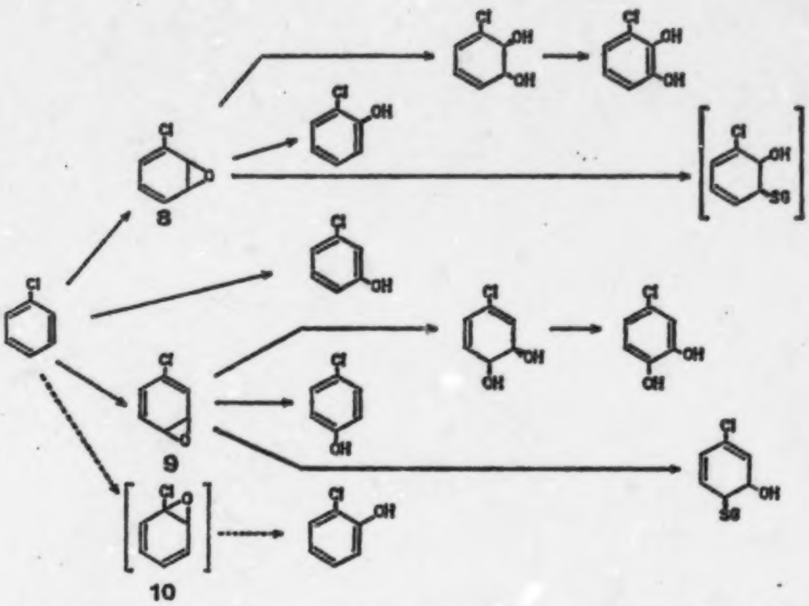
E.6. The Mechanisms of Metabolism

Gardner et al. (319) gave evidence that the principal mechanism of metabolism of chlorobiphenyls was via the formation of an arene oxide (epoxide) intermediate. This has been confirmed for 4-chlorobiphenyl by Safe et al. (325). They used material labelled with deuterium at the 4'-position to show that metabolism in rabbits proceeded via the 3',4'-epoxide, followed by rearrangement to form the 4'-hydroxy- (or to a smaller extent the 3'-hydroxy) derivative. The general importance of arene oxides as intermediates in the metabolism of aromatic compounds has been reviewed by Jerina and Daly (326). One of their examples, the metabolism of chlorobenzene, is shown in Figure E.10. Arene oxides appear to be formed directly by microsomal enzymatic oxidation and then to be rearranged (the "NIH" shift") to



Hydroxylations of polychlorinated biphenyls in the rat
(Main positions are indicated by arrows)

Figure E.9 (from ref. 311)



Mammalian metabolism of chlorobenzene (*50*) to phenols, dihydrodiols, catechols, and glutathione conjugates. Oxides are proposed as intermediates in the formation of 2- and 4-chlorophenol, while the formation of 3-chlorophenol is proposed to occur directly. The structure of the glutathione conjugate derived from **8** is a tentative formulation. Evidence for formation of the oxide **10** has not been obtained. On isomerization, it would be expected to yield 2-chlorophenol.

Figure E.10 (from ref. 326)

E-13c

Table D8 (from ref. 199)

—Histopathologic findings in the liver of male dd mice treated with isomers of benzene hexachloride (BHC) and technical polychlorinated biphenyls (PCBs) for 24 weeks

BHC or PCBs* in diet (ppm)	Effective number of mice†	Histopathology of liver			Liver nodules (percent)	
		Oval cells	Bile-duct proliferation	Cellular hypertrophy	Nodular hyperplasia	Hepatocellular carcinoma
α-BHC(250)	30	±	±	+++	23 (76.7)	8 (26.7)
α-BHC(250) + PCBs-5(250)	26	+	+	+++	21 (80.8)	15 (57.6)
α-BHC(100)	26	−	−	+	0 —	0 —
α-BHC(100) + PCBs-5(250)	25	±	±	++	8 (32.0)	1 (4.0)
α-BHC (50)	28	−	−	−	0 —	0 —
α-BHC (50) + PCBs-5(250)	30	−	−	++	9 (30.0)	2 (6.7)
β-BHC(250)	26	−	−	+	0 —	0 —
β-BHC(250) + PCBs-5(250)	29	±	±	+++	16 (55.2)	6 (20.7)
β-BHC(100)	26	−	−	−	0 —	0 —
β-BHC(100) + PCBs-5(250)	30	−	−	++	5 (16.7)	1 (3.3)
β-BHC (50)	28	−	−	−	0 —	0 —
β-BHC (50) + PCBs-5(250)	29	−	−	+	0 —	0 —
γ-BHC(250)	26	−	−	±	0 —	0 —
γ-BHC(250) + PCBs-5(250)	28	−	−	+	0 —	0 —
γ-BHC(100)	28	−	−	−	0 —	0 —
γ-BHC(100) + PCBs-5(250)	30	−	−	+	0 —	0 —
γ-BHC (50)	28	−	−	−	0 —	0 —
γ-BHC (50) + PCBs-5(250)	27	−	−	+	0 —	0 —
PCBs-5(250)	20	±	±	+	0 —	0 —
Controls	20	−	−	−	0 —	0 —

*PCBs-5, Kanechlor 500.

†Mice dying during the experiment were not included.

form phenols, or to be hydrated to form dihydrodiols (326). In the case of chlorobiphenyls, the data in Figure E.9 suggest that arene oxides tend to form preferentially in the 3,4-position where possible.

The formation of arene oxides requires the presence of adjacent unsubstituted carbon atoms and is therefore more favored for the less chlorinated mixtures. Isomers without adjacent unsubstituted positions can be hydroxylated directly (323), although more slowly. Safe *et al.* (325) showed that the formation of the dihydroxy derivative proceeded by hydroxylation of the monohydroxy derivative, rather than directly from the arene oxide. Other pathways of metabolism for hexachlorobiphenyls without adjacent unsubstituted positions are shown by the work of McKinney *et al.* in chickens (29, 310).

E.7. Formation of CDFs by Metabolism

In addition to the demonstration by McKinney *et al.* (29, 310) that penta- and hexa-CDFs can be produced by metabolism in chickens, Curley *et al.* (327) gave evidence that tetra-CDFs may be formed by metabolism in rats. They isolated a substance from the urine of rats exposed to Aroclor 1254 in the diet with a molecular weight of 304 and an isotopic cluster of 4 chlorine atoms. This matches tetra-CDF but was not positively identified as such. They found evidence of small quantities of the same material in the Aroclor mixture fed to the rats, but the material was much more prominent in the urine of the rats (327).

E.8. Toxicity of Metabolites

Metabolism of PCBs has often been regarded as detoxification, and indeed ultimately is so when the metabolites are conjugated and excreted. However, some of the primary metabolites may be more toxicologically significant than the parent materials. The CDFs are potentially much more toxic than the parent chlorobiphenyls, even if their production is only a minor metabolic pathway (2). Yamamoto and Yoshimura (312) reported that the acute toxicity of the primary metabolite of 2,4,3',4'-tetraCB is 5 times higher than that of the parent compound.

The formation of arene oxides as intermediates in metabolism is of even greater significance. Arene oxides are chemically active compounds which "react

> readily with a variety of nucleophiles, including such cellular macromolecules as DNA, RNA, and protein. Thus, arene oxides have become prime candidates for the 'bioactivated intermediates' responsible for the binding of aromatic compounds to biopolymers within the cell. Since the toxic, carcinogenic, and mutagenic effects of aromatic compounds often correlate with the extent of this binding, arene oxides are now strongly implicated as causative agents in producing these effects." (326).

Thus, for example, the K-region epoxides of polycyclic aromatic hydrocarbons are believed to be the active primary mutagenic and carcinogenic agents (279, 326). The same is likely to be true for PCBs (325, 319, 310, 29, 328). Indeed, Allen and Norback have shown that PCBs are strongly associated with protein and nucleic acid fractions of cells, and that 20% of the metabolites of a tetra-CB are bound to microsomal protein and RNA in a non-extractable form (69). In the words of Matthews and Anderson (328):

"Thus, we are given a dilemma. Those PCBs which can be metabolized and excreted may be metabolized via a carcinogenic intermediate and those PCBs which are not readily metabolized have an extremely long biological half-life. Several researchers have provided evidence that the PCBs may be carcinogenic (ref. 14-16). On the other hand, Vos *et al*. (ref. 17) have shown the very slowly metabolized hexachlorobiphenyl used in this study to be acnegenic, to cause liver damage, and to induce hepatic porphyria. It is not yet known if it is the parent PCBs or their metabolites which account for the primate reproductive failures described by Allen (ref. 18). The only way that we are going to establish which of the PCBs or their metabolites are carcinogenic or are going to cause any of the other toxicological problems and at what levels of exposure the problems are likely to arise is through systematic pharmacokinetic studies which will allow us to extrapolate the results of chronic low dose environmental exposures from laboratory animals to man. Until such data is available it is my opinion that every effort should be made to avoid environmental contamination by any type of PCBs" (328).

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